ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 53, Art. 3. Pages 461–752

Editor
Roy Waldo Miner

Associate Editor
B. J. Henegan

THE GROWTH, REPLACEMENT, AND TYPES OF HAIR

BY

J. B. Hamilton and A. E. Light (*Conference Chairmen*), P. Alexander, B. L. Baker, R. S. Bear, G. W. Bissell, R. J. Block, E. O. Butcher, O. H. Duggins, F. Ellinger, N. C. Foot, S. M. Garn, A. Giroud, M. H. Hardy, L. P. Herrington, C. W. Laymon, C. P. Leblond, A. A. Liebow, R. J. Myers, C. R. Noback, J. P. Parnell, E. L. Reynolds, H. J. Rugo, W. F. Storey, L. W. Thigpen, M. Trotter, and S. B. Wolbach

Consulting Editor
J. B. Hamilton

NEW YORK
PUBLISHED BY THE ACADEMY
March 27, 1951

THE NEW YORK ACADEMY OF SCIENCES
(Founded in 1817)

OFFICERS, 1951

President
WILLIS A. GIBBONS

Vice-Presidents
JOHN RAY DUNNING WALTER S. ROOT

Recording Secretary
HARRY A. CHARIPPER

Corresponding Secretary
JAMES B. ALLISON

Treasurer
DONALD M. BENJAMIN

Editor
ROY WALDO MINER

Elected Councilors
1949–1951
EDITH QUIMBY JOHN TEE VAN W. G. BYWATER
1950–1952
E. H. NORTHEY JOHN A. AESCHLIMANN ERICH M. SCHLAIKJER
1951–1953
EDWIN J. DEBEER DAVID B. STEINMAN OSKAR P. WINTERSTEINER

Finance Committee
ROBERT F. LIGHT, *Chairman* GORDON Y. BILLARD HARDEN F. TAYLOR

Executive Director
EUNICE THOMAS MINER

SECTION OF GEOLOGY AND MINERALOGY
SIDNEY PAIGE, *Chairman* M. HALL TAYLOR, JR., *Secretary*
SECTION OF BIOLOGY
ELI D. GOLDSMITH, *Chairman* CHARLES W. MUSHETT, *Secretary*
DIVISION OF MYCOLOGY
BENJAMIN M. DUGGAR, *Chairman* WILLIAM J. ROBBINS, *Secretary*
SECTION OF PSYCHOLOGY
THOMAS N. JENKINS, *Chairman* N. L. HOOPINGARNER, *Secretary*
SECTION OF ANTHROPOLOGY
PAUL FEJOS, *Chairman* JANE BELO, *Secretary*
SECTION OF PHYSICS AND CHEMISTRY
CHARLES O. BECKMANN, *Chairman* FRANK BRESCIA, *Secretary*
SECTION OF OCEANOGRAPHY AND METEOROLOGY
WILLIAM L. DONN, *Chairman* RICHARD J. ROMMER, *Secretary*
SECTION OF MATHEMATICS AND ENGINEERING
ABRAHAM SLAVIN, *Chairman* SEBASTAIN B. LITTAUER, *Secretary*

These Sections and the Division hold meetings regularly, one evening each month, during the academic year, October to May, inclusive.

Two-day conferences also are held at irregular intervals. All meetings are held at the building of The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, New York.

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 53, Art. 3. Pages 461–752

March 27, 1951

Editor
Roy Waldo Miner

Associate Editor
B. J. Henegan

THE GROWTH, REPLACEMENT, AND TYPES OF HAIR

Conference Chairmen: J. B. Hamilton and A. E. Light
Consulting Editor: J. B. Hamilton

CONTENTS

Introduction. *By* J. B. Hamilton and A. E. Light 463
Histological Structure of Hair, with a Brief Comparison to Other Epidermal Appendages and Epidermis Itself. *By* C. P. Leblond 464
Morphology and Phylogeny of Hair. *By* C. R. Noback 476
Hair Pattern and Distribution in Mammals. *By* J. P. Parnell 493
Types and Distribution of the Hair in Man. *By* S. M. Garn 498
Development of the Pilary System and the Replacement of Hair in Mammals. *By* E. O. Butcher 508
The Hair Cycle of the Mouse and its Importance in the Study of Sequences of Experimental Carcinogenesis. *By* S. B. Wolbach 517
Measurement of the Rate of Proliferation of Epidermis and Associated Structures. *By* W. F. Storey and C. P. Leblond 537
The Development of Pelage Hairs and Vibrissae from Skin in Tissue Culture. *By* M. H. Hardy 546
Regeneration and Rate of Growth of Hairs in Man. *By* R. J. Myers and J. B. Hamilton 562
Changes in Morphology of Hair During Childhood. *By* O. H. Duggins and M. Trotter 569
The Appearance of Adult Patterns of Body Hair in Man. *By* E. L. Reynolds 576
Quantitative Measurement of a Secondary Sex Character, Axillary Hair. *By* J. B. Hamilton 585
The Role of the Piliary System in Mammals and its Relation to the Thermal Environment. *By* L. P. Herrington 600
Chemical Classification of Keratins. *By* R. J. Block 608
The Keratinization of Epidermis and its Derivatives, Especially the Hair, as Shown by X-Ray Diffraction and Histochemical Studies. *By* A. Giroud and C. P. Leblond 613
The Results of X-Ray Diffraction Studies on Keratin Fibers. *By* R. S. Bear and H. J. Rugo 627
The Examination of Hair Under the Polarizing Microscope. *By* S. M. Garn 649
Changes in the Physical Properties of Wool Fibers Produced by Breaking Hydrogen Bonds with Lithium Bromide Solutions. *By* P. Alexander 653

This series of papers is the result of a Conference on *The Growth, Replacement, and Types of Hair*, held by the Section of Biology of The New York Academy of Sciences, February 10 and 11, 1950.

Publication made possible by grants-in-aid from the Bristol-Meyer Company; Raymond Laboratories, Inc.; Colgate-Palmolive-Peet, Inc.; The Toni Company; Lever Brothers, Inc.; R. B. Semler, Inc.; The Mennen Company; Charles Marchand and Company; Chesebrough Mfg. Company, Inc., Parker-Herbex Corporation; Procter and Gamble, Inc.; John M. Breck, Inc.; Harris Research Laboratories, Inc.; Gillette Safety Razor Company, Inc.; and the General Funds of The New York Academy of Sciences.

Inheritance—Distribution, Pattern, Quality, and Quantity. *By* L. W. THIGPEN 674
Effects of Ionizing Radiation on Growth and Replacement of Hair. *By* F. P. ELLINGER 682
Effects Produced on Hair by Atomic Bombs. *By* A. A. LIEBOW 688
The Relationship of the Adrenal, Thyroid, and Pituitary Glands to the Growth of Hair. *By* B. L. BAKER 690
Patterned Loss of Hair in Man: Types and Incidence. *By* J. B. HAMILTON 708
Patterned Loss of Hair in Man: Pathogenesis and Prognosis. *By* A. E. LIGHT 729
The Non-Patterned Alopecias. *By* C. W. LAYMON 735
Hirsutism. *By* G. W. BISSELL 742
The Role of the Piliary System in Cancer of the Skin. *By* N. C. FOOT 749

Copyright, 1951, by The New York Academy of Sciences

INTRODUCTION

The Conference on "The Growth, Replacement and Types of Hair" was organized with the idea of assembling known facts pertaining to many phases of the growth and development of hair, with special emphasis on new and hitherto unpublished research work.

Certain aspects, such as pigmentation, bacteriological effects, and the cosmetics of hair waving were purposely omitted in order to keep the conference within reasonable limits.

Acknowledgments should be made to the Council of the New York Academy of Sciences, to Mrs. Eunice T. Miner, Executive Director of the Academy, and to Dr. E. D. Goldsmith, Chairman of the Section of Biology, for making this conference possible. The assistance of Drs. C. R. Noback, E. O. Butcher, R. J. Block, and others, in various phases of this undertaking, is noted with appreciation.

JAMES B. HAMILTON, *State University Medical Center at New York City, Brooklyn, N. Y.*

and

AMOS E. LIGHT, *The Wellcome Research Laboratories, Tuckahoe, N. Y.*

HISTOLOGICAL STRUCTURE OF HAIR, WITH A BRIEF COMPARISON TO OTHER EPIDERMAL APPENDAGES AND EPIDERMIS ITSELF

By C. P. Leblond

Department of Anatomy, McGill University, Montreal, Canada

Hair plays a fundamental role in the maintenance of body temperature in mammals. Man, whose hair is no more than a symbol of strength or beauty, uses animal hair, such as fur or wool, to achieve the same end. The remarkable properties of hair—heat-retaining capacity, durability, and resiliency—are due to its high content of a sulfur-containing protein, keratin.*

Keratin is also present in epidermis and epidermal appendages, nails, claws, horns, feathers, *etc.* In all of these structures, the superficial layers, which are impregnated with keratin, arise from the transformation of the cells of the so-called Malpighian layers. Thus, Malpighian and keratinized layers make up the epidermis (FIGURE 1), the hair (FIGURE 2), and the other appendages.

The Malpighian layers present in all these structures take the form of stratified squamous epithelium, the basal cells of which are separated from the underlying connective tissue by a thin layer of fairly distinct reticular fibers (as can be seen, for instance, at the limit between epidermis and derma, FIGURE 3). In areas of low biologic activity, such as the outer root sheath of the hair, the reticular fibers fuse to form a continuous basement membrane (FIGURE 4), whereas in areas of high activity, such as the Malpighian layer at the base of the hair follicle, reticular fibers are scarce or even absent (FIGURE 5). These facts suggest a relationship between the thinness of the reticular layer and the intensity of the metabolic exchanges across such a layer from the subjacent connective tissue. The activity of the Malpighian layer is related to that of the subjacent tissue in many other ways. Thus it can be shown that the connective tissue beneath actively growing Malpighian layers, *e.g.*, the hair papilla, is highly vascularized, contains many nuclei (FIGURE 7), and is rich in phosphatase[4] and metachromatic substances,[5] in contrast to the derma in general.

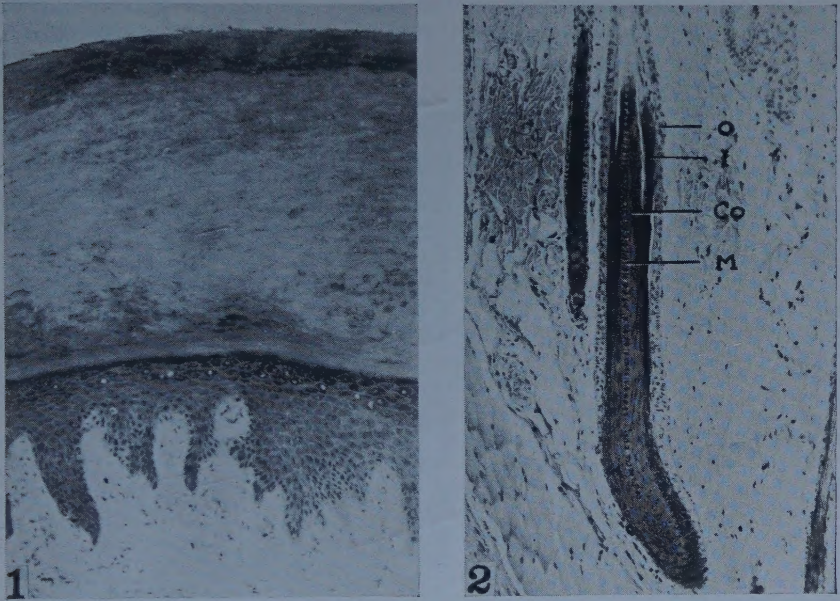
The keratinized layers are formed by cells having a fairly homogeneous appearance and in which most cytological details are no longer recognizable. The keratin content is responsible for their low solubility and high resistance to many chemical agents.

On the basis of histological appearance and chemical constitution, two types of keratin, namely, soft and hard, can be distinguished.

Soft Keratin. The structures containing soft keratin, typically the epidermis, show a characteristic transition between the Malpighian and keratinized layers. The cells in the upper part of the Malpighian contain irregular basophilic granules which more or less fill up the cytoplasm. This has led to the convention of subdividing the Malpighian layers into a nongranular *stratum germinativum* or germinal layer and a granular *stratum granulosum* or granular layer (FIGURE 1). Between the outer limit of the *stratum granulosum* and the keratinized layers there is usually a narrow region in which the cells are transparent and without visible cytological detail. This region is known as the glassy layer or *stratum lucidum*. External to this is an area containing more opaque material, known as the keratinized layer or *stratum corneum* (FIGURE 1). Unna described the cells of the layer as having a thick hard wall and a fluid core rich in fats. It is often possible to recognize in paraffin sections the center of the cells, where nuclear remnants may be seen. It is probable that this fluid cell center renders the epidermal keratin somewhat supple and malleable, and this characteristic leads to its name of "soft keratin."

---

* Most of the available material on the histology of the hair is summarized by Branca[1] and von Hoepke.[2] More recent information is presented by Trotter.[3]



FIGURES 1 AND 2.

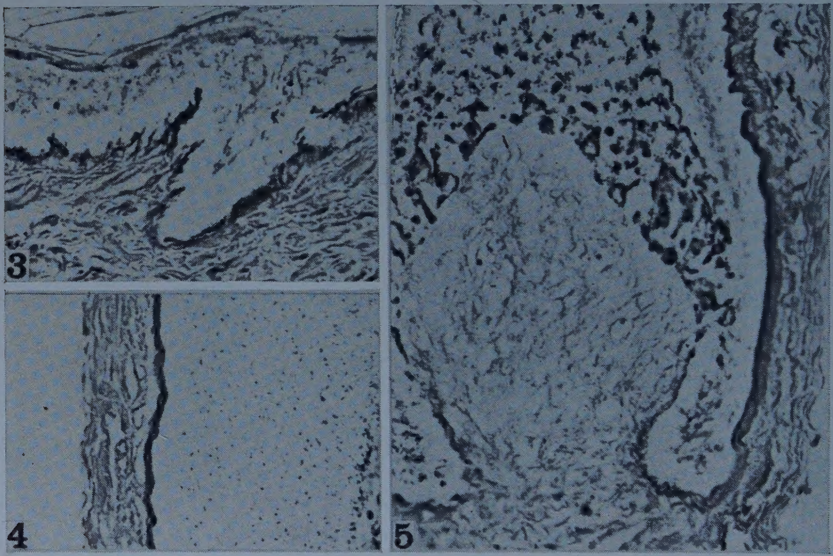
FIGURE 1. Cross section of epidermis from the palm of the hand of a monkey. Hematoxylin-eosin. Low magnification.

From the bottom to the top, it is possible to see (1) the papillary zone of the derma, (2) the germinal layer and the darker granular layer, which together make up the living or Malpighian zone of the epidermis, (3) the small glassy layer and the large horny layer, which together make up the non-living or keratinized zone of the epidermis. It may be noted that the nuclei, as such, are no longer recognizable in the keratinized layers, but the nuclear area is frequently visible as an irregularly stained spot. (Slide of McGill collection, prepared by Dr. J. Gross.)

FIGURE 2. Section through derma showing the hair follicles of a small (left) and large (right) hair in the rat. Hematoxylin-eosin. The letters used to label this photograph are: O, outer root sheath; I, inner root sheath; Co, cortex of the hair (transition zone); M, medulla of the hair. The larger hair follicle shows from the bottom of the figure to the top: (1) the Malpighian layers, in which the cells producing the internal root sheath can be recognized by their darker stain; (2) in the middle portion of the figure, the area of transition between the Malpighian layers and the hair shaft (keratogenous zone). This area stains darkly. The cells of the medulla may be seen in its central portion. On the outside, the inner root sheath stands out as a still darker structure, while the outer root sheath is pale. Note that the outer root sheath does not extend to the end of the hair follicle, especially on the left hand side; (3) at the top of the picture, the shaft of hair which appears as an unstained area continuous with the dark transition zone. At this level, the inner root sheath is in the process of desquamating. The outer root sheath shows no apparent changes. (Section prepared by Miss W. F. Storey.)

The process of soft keratinization is indicated histologically by the presence of the granular and glassy layers, chemically by a low or only moderate amount of sulfur, and physiologically by instability, as evidenced by a spontaneous, continuous desquamation from the surface.[6, 7]

Hard Keratin. Hard keratin is a particularly tough and firm material found in many of the appendages. On histological examination, the structures containing hard keratin show a gradual transition from the Malpighian to the keratinized layers without the sharply demarcated zones found in structures producing soft keratin. This area of gradual transition, which was named the "keratogenous zone" by Giroud and Bulliard,[6] can be found in all appendages (nails, feathers, horns, hooves, hair [FIGURES 6 and 18], *etc.*) Chemically,* hard keratin has a high sulfur content and is rather unreactive, a fact which may explain why it is not stained by routine histological dyes, in contrast to soft keratin. Physiologically, hard keratin does not desquamate from the surface.



FIGURES 3–5.

Sections of human skin (scalp). Silver stain for reticular fibers. High magnification.

FIGURE 3. The limit between the epidermis (upper half) and derma (lower half) is indicated by black-staining reticular fibers that are often separated from one another.

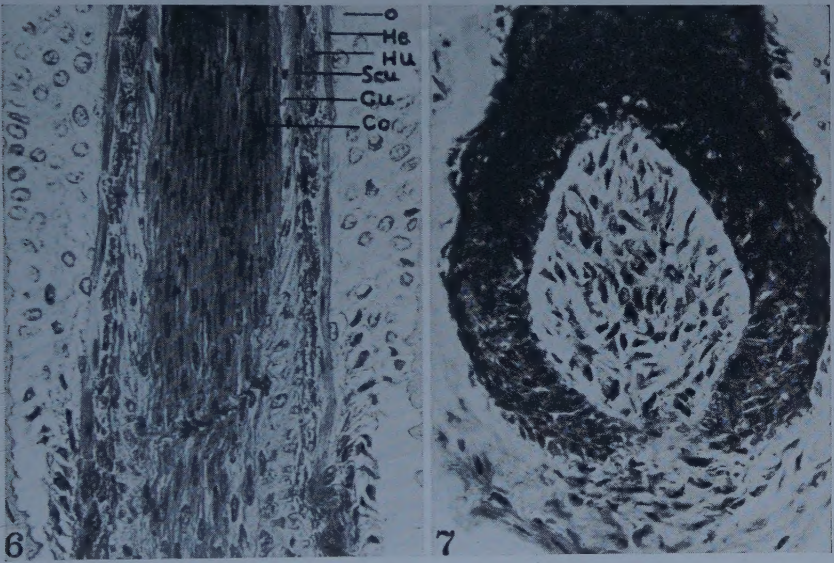
FIGURE 4. The limit between the outer root sheath of a hair (right hand side) and the derma (left hand side) is indicated by a thick black basement membrane.

FIGURE 5. Hair root with papilla. On the right hand side, the black line corresponds to the basement membrane of the outer root sheath, which thins out as it folds around the edge of the papilla. No reticular fibers are visible between the Malpighian layers (recognizable by the black granules of pigment) and the connective tissue of the papilla.

Keratinization of Hair and Other Epidermal Appendages. Several components of the hair root (FIGURES 2, 8–13) produce soft keratin. Thus, the external root sheath, which is continuous with the epidermis, has granular, glassy, and cornified layers at least in the upper part of the hair root (*i.e.*, near the opening of the sebaceous glands), while the lower part of this same external root sheath appears to be composed of dormant cells, which are producing little or no soft keratin. The base of the hair root consists of Malpighian cells, which form structures which grow parallel to the hair, namely, the internal root sheath on the outside and the medulla of the hair in the center. Both of these structures have a more or less distinct granular layer (FIGURES 10 and 11), as well as areas of desquamation (FIGURE 13) and therefore must be producing soft keratin. Thus, the empty central canal often present in a fully grown hair can be explained as the result of desquamation of the medullary cells.

---

* Histochemical and other differences between soft and hard keratin will be reviewed in detail in another paper.[8]



FIGURES 6 AND 7.

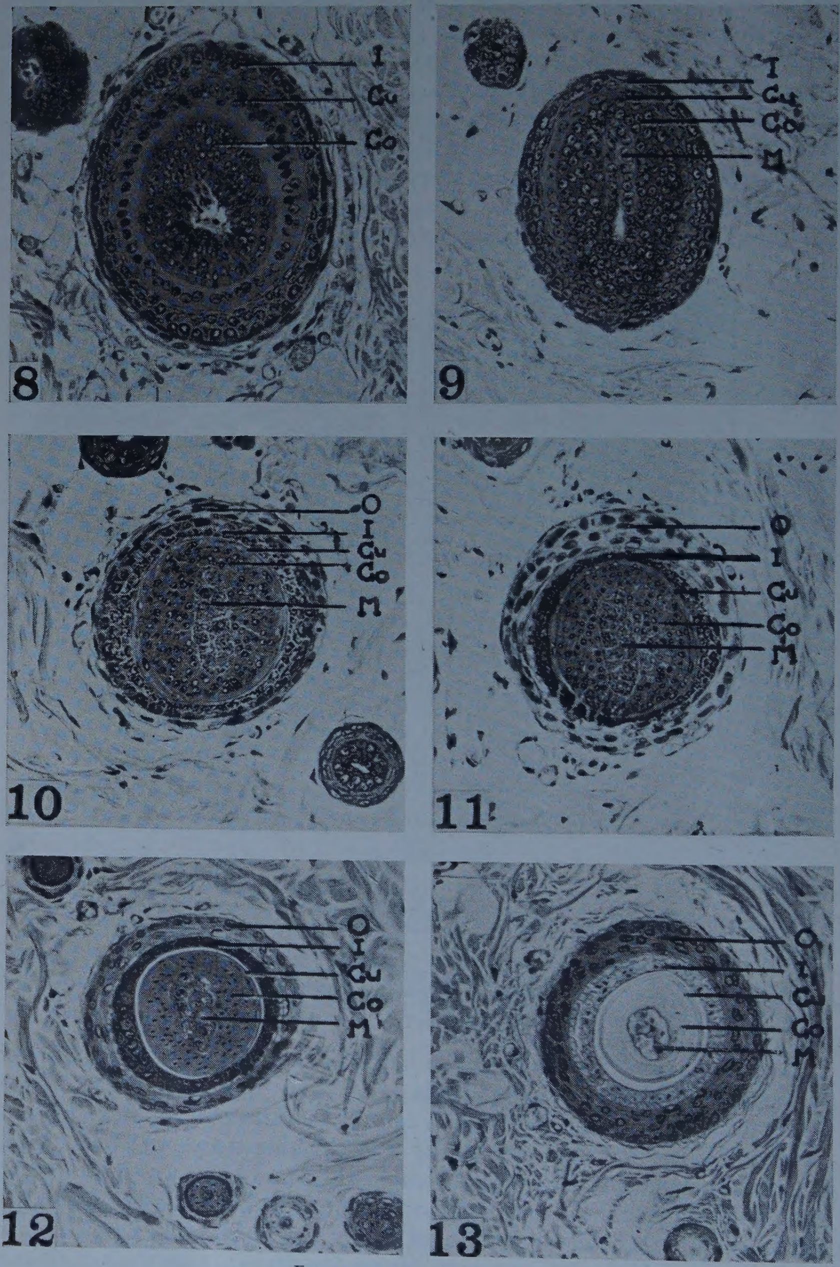
Human hair (scalp) in longitudinal section.

FIGURE 6. Keratogenous zone between Malpighian layers (bottom) and cortex (above). (Masson trichrome.) O, outer root sheath; He, Henle layer, Hu, Huxley layer, and Scu, sheath cuticle—all of the inner root sheath; Cu, hair cuticle; and Co, hair cortex. The axial orientation of the cells of the cortex with their tonofibrils is indicated.

FIGURE 7. Papilla of the hair. Iron hematoxylin. Note the rather large number of nuclei in the connective tissue of the papilla and immediately below it. In contrast, the connective tissue on the side of the hair contains very few cells. Note also the absence of medulla in this hair, as well as the abundance of pigment (Slide of McGill collection, prepared by Dr. J. Gross.)

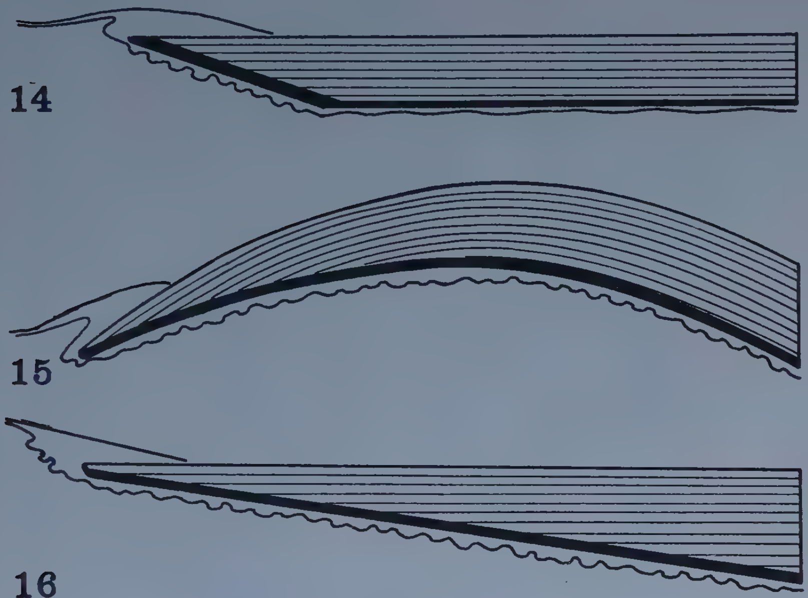
However, most of the Malpighian cells of the hair root produce the hair cortex, or hair proper, as well as the thin covering cuticle (FIGURES 8–13, 18). Both of these structures are composed of hard keratin. Between the Malpighian zone and the fully formed hair, there is the gradual transition or keratogenous zone, characteristic of structures producing hard keratin (FIGURES 6 and 18). Structural details are illustrated in FIGURES 2, 6, and 8–13.

In some appendages, such as nails (FIGURE 14), claws (FIGURE 15), and horns (FIGURE 16), the formation of a solid mass of hard keratin is simple



FIGURES 8–13 (*see facing page*).

and direct, whereas, in the feather (FIGURE 17), the complexity of the picture is comparable to that of the hair (FIGURE 18). Thus, the bulb of the feather is surrounded by an external root sheath continuous with the epidermis. Also, a cuticle and cortex are present, as well as a broad central canal which is bridged by several lamellae of keratin, and may be compared to the hair medulla. The cuticle forms a definite outer wall, which kera-



FIGURES 14–16.

Longitudinal sections of nail (FIGURE 14), claw (FIGURE 15), and horn (FIGURE 16). The hard keratin of these structures is indicated by parallel lines. The heavy lines indicate the keratogenous zones. The Malpighian layers are outlined below the keratogenous zones. On the left of each diagram, the soft keratin of the eponychium may be seen. (Redrawn by Miss L. Cimon from Giroud and Bulliard.[9])

FIGURES 8–13. (*see opposite page*).

Cross sections of a hair from the back skin of an adult male rat, at various levels. The letters used to label these photographs are as follows: O, outer root sheath; I, inner root sheath; Cu, cuticle of the hair; Co, cortex of the hair; and M, medulla. It must be emphasized that the same letters were used to label the Malpighian and keratinized portions of each one of these structures. Since the hair comes from an albino rat, no pigment hides structural details.

FIGURE 8. Cross section through the papilla showing the Malpighian layers of inner root sheath, cuticle, and cortex.

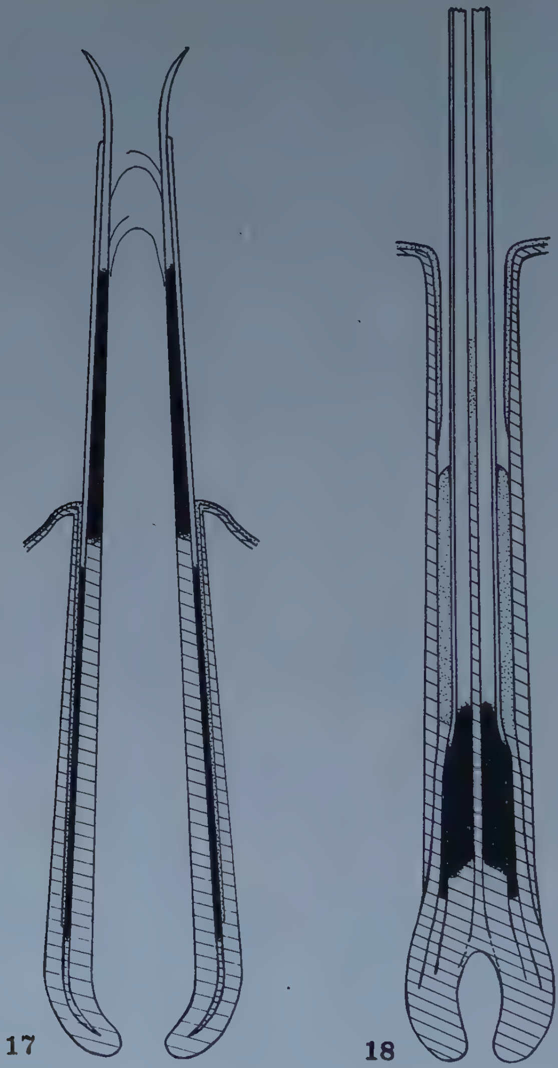
FIGURE 9. Cross section through the upper part of the papilla showing the Malpighian layers producing the inner root sheath, cuticle, cortex, and medulla of the hair.

FIGURE 10. Cross section through the transition zone between the Malpighian and keratinized layers of the hair cortex (keratogenous zone). Note, from outside in, the first appearance of the Malpighian cells of the outer root sheath, the Malpighian cells of the inner root sheath, the transition zone of the cuticle, and that of the cortex, and the granular zone of the medulla.

FIGURE 11. Same as FIGURE 6, except that the inner root sheath is cut at a level corresponding to the granular layer of most of its cells.

FIGURE 12. Cross section through the top of the keratogenous zone of the hair cortex. At this level, the inner root sheath is keratinized. The nuclei of the cuticle and cortex are in the process of disappearing. Compare to FIGURE 2.

FIGURE 13. Cross section of the hair just before it goes through the epidermis. Note that the cuticle and cortex show no nuclei and do not take up histological stains. Both the inner root sheath and the medulla are undergoing desquamation at this level. (Section prepared by Miss W. F. Storey.)



FIGURES 17 AND 18.

Longitudinal sections of a down feather (FIG. 17) and a hair (FIG. 18) showing in black the keratogenous zones. The Malpighian layers are cross-hatched. The soft keratin is stippled. The hard keratin is white.

FIGURE 17. The feather consists of two imbricated tubes of hard keratin, a thin short one which keratinizes at a low level in the bulb of the feather, and a thicker one which makes up the feather itself and keratinizes at a higher level. The central canal, or medulla, has a few fine transverse lamellae and may correspond to the hair medulla.

FIGURE 18. The hair shows cuticle, cortex, and medulla, surrounded by inner and outer root sheath. (Re-drawn by Miss L. Cimon from Giroud and Bulliard,[9] with modifications.)

tinizes deeper in the feather root than the cortex proper. The cortex itself is formed by a thick layer of hard keratin, which gradually splits into rays in a specific manner for each type of feather. Thus, the cortex of the down feathers consists of imbricated oblique rings, which spread out into barbs as they extend some distance from the root.[6]

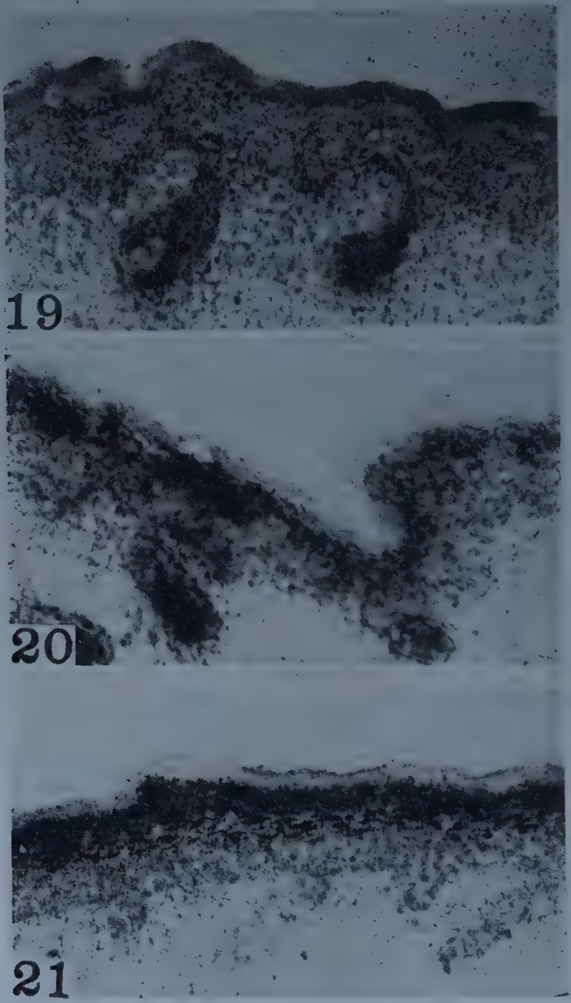
Characters Common to the Two Types of Keratinization. From the histological viewpoint, the epidermis and its derivatives possess several similarities. Two of these will be emphasized here, namely, the outward motion of the cells, and the formation of keratin from a structural precursor which is believed to be the tonofibril.

In the Malpighian layer of the epidermis and its derivatives, a number of cells are dividing by mitosis. These mitoses occur in the lowermost layers. Their number and constancy are such that they must produce an outward motion of the cells.[1, 10] In the course of this outward motion, the cells keratinize, in either the soft or hard keratinization pattern, depending on their location. The sequence may be illustrated in sections of the skin taken at various time intervals after a single injection of radioactive carbonate (C^{14}) into a newborn rat. At two hours after administration, a slight amount of radioactivity may be detected by the autographic method[11] throughout the germinal layer (FIGURE 19). Twenty-four hours after injection, a fairly large amount of radioactivity is present in the region of the granular layer (FIGURE 20). Three days after injection, the radioactivity may be found in the keratinized layers (FIGURE 21). While the C^{14}-containing substances responsible for these reactions have not been identified, they must be sufficiently insoluble not to be extracted by the reagents used during the histological procedure. Presumably, a number of organic compounds were synthesized in the Malphigian layers from the circulating labeled carbonate and were retained in the process of cornification, possibly in the form of keratin itself.

In the hair follicle, mitoses occur only in periods of activity (see Butcher's paper, page 508). In such periods, mitoses become quite numerous throughout the lower portions of the Malpighian layers. These mitoses result in an outward motion along the hair axis of most components of the follicle. In the course of this motion, the cells of the medulla and inner root sheath (soft keratin structures) desquamate. Thus, only cortex and cuticle, both of which are hard keratin structures, persist to form the emerging hair.

The second feature of interest is the information obtained by histological methods regarding the structure of the two types of keratin. One of the principal features of both keratins is their resistance to the action of physical and chemical agents. Physical resistance may be estimated by measuring the weight necessary to produce rupture. In the case of soft keratin, Giroud and Champetier[12] stated that the Malpighian layers were almost as resistant to rupture as the soft keratin of the cornified layers of the epidermis. The inference is that the Malpighian layers contain a keratin precursor which already possesses the strength characteristic of keratin itself. Histological study reveals that the one structure common to all Malpighian and cornified layers is the tonofibril, which is thus probably the cytological representation of keratin, as well as its precursor in Malpighian layers.

In the epidermis, the Malpighian cells clearly show a large number of tonofibrils in their cytoplasm (FIGURE 22). These tonofibrils are perpendicular to the skin surface in the *stratum germinativum*, but become more and more nearly parallel to the surface as the cells move outward.



FIGURES 19–21.

Radio-carbon autographs of the epidermis of new-born rats, sacrificed at various time intervals after injection of 50 microcuries of C^{14} as sodium carbonate. These preparations were obtained by cutting six μ sections of the formalin-fixed epidermis. These sections were then coated in the dark with photographic emulsion melted from Eastman Kodak Medium lantern slides. They were then kept in a light-tight container at 1–2°C. for 18 months to allow the radioactive carbon of the sections to bombard the emulsion. The slides were then taken through developer and fixer in order to render visible as black granules the portions of the emulsion affected by the radiocarbon. The stippling of the background corresponds to photographic fog and has no bearing on the problem.

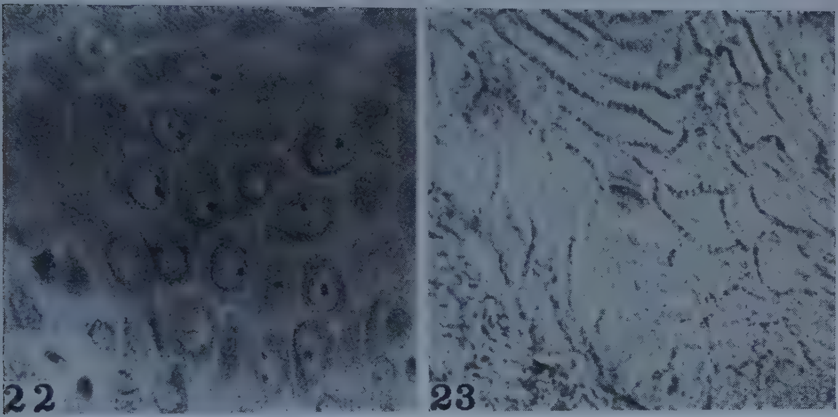
FIGURE 19. Two hours after injection of C^{14}. A moderate reaction is present in the Malpighian layers of the epidermis. No reaction is present in the keratinized layer. In the derma, two hair buds show a fairly intense reaction.

FIGURE 20. Twenty-four hours after injection of C^{14}. A fairly intense reaction is visible on the granular layer separating the rest of the Malpighian layer (showing only a slight reaction) from the cornified layers (showing no reaction). Three hair follicles show a fairly intense reaction.

FIGURE 21. Seventy-two hours after injection of C^{14}. The Malpighian layers, including the granular layer, show little or no reaction, but the deeper portion of the cornified layer shows an intense reaction appearing as a fairly regular dark band. It is probable that the radioactive material, moving up with the cells of the skin towards the outside, is a tissue protein (possibly keratin itself), as it is not extracted from the sections by watery and fatty solvents.

For some unexplained reason, the reaction of the hair follicles has decreased or disappeared at this time. (Possibly the radioactive material in the hair follicles was used in local oxidations and eliminated as residues, such as carbon dioxide.)

These fibrils are found close to the cell walls rather than uniformly dispersed throughout the cytoplasm, as they are in the deepest layers, a fact which may help to explain their changed direction. It has generally been assumed that single fibrils pass through several cells. This continuity of fibrils may help in holding the cells together and may be important in explaining the resistance of Malpighian layers to rupture.[12] On the other hand, several authors suggested that the tonofibrils do not run from cell to cell but extend only slightly beyond the cell membrane through the intercellular space, where they would join with a tonofibril from a neighboring cell.[2] This would offer an explanation for the nodular appearance (nodules of Bizzozero) of the tonofibrils in the intercellular spaces. An incidental observation was not in agreement with this last theory. While investigating the distribution of polysaccharides by the method of Hotchkiss,[13] it was noted that the tonofibrils themselves did not stain by this technique, but the nodules of Bizzozero did show a reaction (FIGURE 23). It was concluded from this observation that the nodules are the result of an accumulation of polysaccharides on the intercellular portions of the tonofibrils, and not the result of the junction, side by side, of two different fibrils. Furthermore, in iron hematoxylin preparations, the continuity of the fibrils was easily recognizable. Therefore, it appears safe to accept the classical opinion that the tonofibrils form a network throughout the Malpighian layers of the epidermis.



FIGURES 22 AND 23.

FIGURE 22. Tonofibrils in the plantar epidermis of the hind feet of the albino rat. Hematoxylin-eosin. High magnification. The intercellular bridges are visible in the center of the picture. (Preparation by Miss W. F. Storey.)

FIGURE 23. Epidermis stained with the periodic acid—fuchsin-sulphurous acid method of Hotchkiss. Only the granules of the intercellular bridges show. Therefore, these granules consist of a substance capable of reacting with periodic acid to produce an aldehyde. It is presumably a polysaccharide. (Preparation by Y. Clermont.)

In the keratinized layers of the epidermis, the tonofibrils have been seen by Weidenreich with the help of staining methods,[14] by Albertini using the phase microscope,[15] and by Panebianco with the polarization microscope.[16]

They may often be recognized in routine sections near broken portions of the cornified layers, where they are seen at the frayed edges of the cells.

In the hair, the fibrils are not clearly visible in the basal cells of the Malpighian layers, but they become definite in the upper portion, and are most pronounced in the keratogenous zone. They are always oriented in a direction parallel to the long axis of the hair shaft, that is, perpendicular to the basal layers (FIGURE 6).

In the keratinized portion of the hair, the individual tonofibrils are not clearly visible in stained preparations, but their persistence from the keratogenous zone is indicated by the lining up of pigment granules and the birefringence, which is first found in the upper portion of the Malpighian layers and persists along the whole length of the hair.[17]

Similarly, in nails, claws, and horns, where the tonofibrils are most easily seen in the keratogenous zone, they are parallel to the general orientation of the finger and the nail itself (FIGURES 14–16).

The universal presence of tonofibrils in the epidermis and the above-mentioned derivatives suggests that the tonofibrils of the Malpighian layers are the precursors of keratin, while those of the cornified portions are actually the histological manifestation of keratin itself.[8]

Conclusion. The Malpighian cells of the *hair follicle* are capable of producing (1) soft keratin structures, namely, the medulla of the hair and the inner root sheath, both of which desquamate before the hair reaches the surface of the epidermis, and (2) hard keratin structures, namely, the cortex and cuticle, which emerge from the epidermis to form the hair itself.

Emphasis is placed on the evolution of epidermal derivatives as a consequence of mitoses in the Malpighian layer, and on the universal presence of tonofibrils in structures undergoing keratinization.

Bibliography

1. BRANCA, A. 1912. Le tégument externe et ses dérivés. Traité d'Anat. humaine, by P. Poirier and A. Charpy. **5:** (fasc. 2): 1.
2. HOEPKE, H. VON 1927. Die Haut. in: W. v. Möllendorff's Handb. d. mikr. Anat. des Menschen. **3** (1): 1. Berlin.
3. TROTTER, M. 1932. The hair. Special Cytology, by E. V. Cowdry. **1** (2): 41.
4. JOHNSON, P. L., E. P. BUTCHER, & G. BEVELANDER. 1945. The distribution of alkaline phosphatase in the cyclic growth of the rat hair follicle. Anat. Rec. **93:** 355.
5. GIROUD, A. Personal communication.
6. GIROUD, A. & H. BULLIARD. 1930. La kératinisation de l'épiderme et des phanères. Genèse des substances soufrées de la kératine. Arch. de morph. (fasc. 29): 1.
7. GIROUD, A., H. BULLIARD, & C. P. LEBLOND. 1934. Les deux types fondamentaux de kératinisation. Bull. d'histol. appliq. à la Physiol. **11:** 129.
8. GIROUD, A. & C. P. LEBLOND. 1951. The keratinization of epidermis and its derivatives, especially the hair, as shown by X-ray diffraction and histochemical studies. Ann. N. Y. Acad. Sci. **53** (3): 613.
9. GIROUD, A. & H. BULLIARD. 1934. Croissance et kératinization des phanères. Arch. Zool. expér. et gén. **34:** 40.
10. STOREY, W. F. & C. P. LEBLOND. 1951. Measurements of the rate of proliferation of epidermis. Ann. N. Y. Acad. Sci. **53** (3): 537.
11. LEBLOND, C. P., W. PERCIVAL, & J. GROSS. 1948. A method for the autographic localization of radio-iodine on stained sections of thyroid gland coated with a photographic emulsion. Proc. Soc. Exp. Biol. & Med. **67:** 74.

12. Giroud, A. & G. Champetier. 1936. Recherches sur les roentgénogrammes des kératines. Bull. Soc. de Chim. biol. **18:** 656.
13. Leblond, C. P. 1950. Distribution of periodic acid-reactive carbohydrates in the adult rat. Am. J. Anat. **86:** 1.
14. Weidenreich, F. 1900. Ueber Bau und Verhornung der menschlichen Oberhaut. Arch. f. mikr. Anat. **56:** 169.
15. Albertini, A. 1945–46. Pflasterepithelizellen im Phasenkontrastbild. Acta anat. **1:** 463.
16. Panebianco, E. 1897. Studio ottico cristallografico della cheratino. Riv. di mineral. e ital. **17:** 17.
17. Schmidt, W. J. 1924. Die Bausteine des Tierkorpers im polarisiertem Lichte. Bonn.

MORPHOLOGY AND PHYLOGENY OF HAIR

By Charles R. Noback*

Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York

Hair is a structure found exclusively in mammals. With this in mind, Oken named the Mammalia, Trichozoa (hair animals), and Bonnet (1892) named them Pilifera (hair bearers).

Of the many aspects of morphology and phylogeny of hair, only four will be discussed. These include (1) the principle of the arrangement of hairs in group patterns, (2) the types of hair and their relation to the principle of the group pattern, (3) a brief analysis of the structural elements of hair and their relation to the types of hair, and (4) the phylogeny of hair, with some remarks on (*a*) the relation of hair to the epidermal derivatives of other vertebrate classes and (*b*) aspects of the phylogeny of the hair and wool of sheep to illustrate that marked differences in hair coats exist between closely related animals.

Hair is the subject of a voluminous literature. Toldt (1910, 1912, 1914, and 1935), Danforth (1925a), Pinkus (1927), Pax and Arndt (1929–1938), Trotter (1932), Lochte (1938), Smith and Glaister (1939), and Stoves (1943a) discuss the problem of mammalian hair in general. Wildman (1940), von Bergen and Krause (1942), and the American Society for Testing Materials (1948) discuss the problem of fiber identification as applied to textiles.

Principle of the Group Pattern of Hairs

In the only extensive survey of the grouping of hair in mammals, DeMeijere (1894) documented the concept of the group pattern of hair (FIGURES 1–6). Unfortunately, the few studies on this phase of the problem since that time have not fully exploited the implications of this concept. DeMeijere concluded that hairs are mainly arranged in groups with the pattern of 3 hairs—with the largest hair in the middle—as the basic pattern. The concept of the basic trio as the primitive condition is accepted as an adequate working hypothesis by Wildman (1932), Galpin (1935), Höfer (1914), Gibbs (1938), Hardy (1946), and others. DeMeijere described 8 patterns: (1) 3 or less hairs behind each scale of the tail (as in the opossum, *Didelphis marsupialis*), (2) more than 3 hairs behind each scale of the tail (as in the rodent, *Loncheres* [Echimys] *cristata*), (3) 3 hairs (as in the back of the marmoset, *Midas rosalia*), (4) more than 3 hairs arranged in a regular pattern with some of greater diameter than others (as in the back hairs of *Loncheres* [Echimys] *cristata* in FIGURE 3), (5) several hairs composed of a number of fine hairs and one coarse hair (as in the back of the dog, *Canis familiaris*, in FIGURE 5D), (6) several hairs composed of a number of fine hairs and one isolated coarse hair (as in the back hairs of the mouse, *Mus decumanus*, in FIGURE 6D), (7) scatterings of fine hairs with no apparent

---

* The author wishes to thank Dr. Margaret Hardy, Division of Animal Health and Production, Sydney, Australia, for her valuable suggestions.

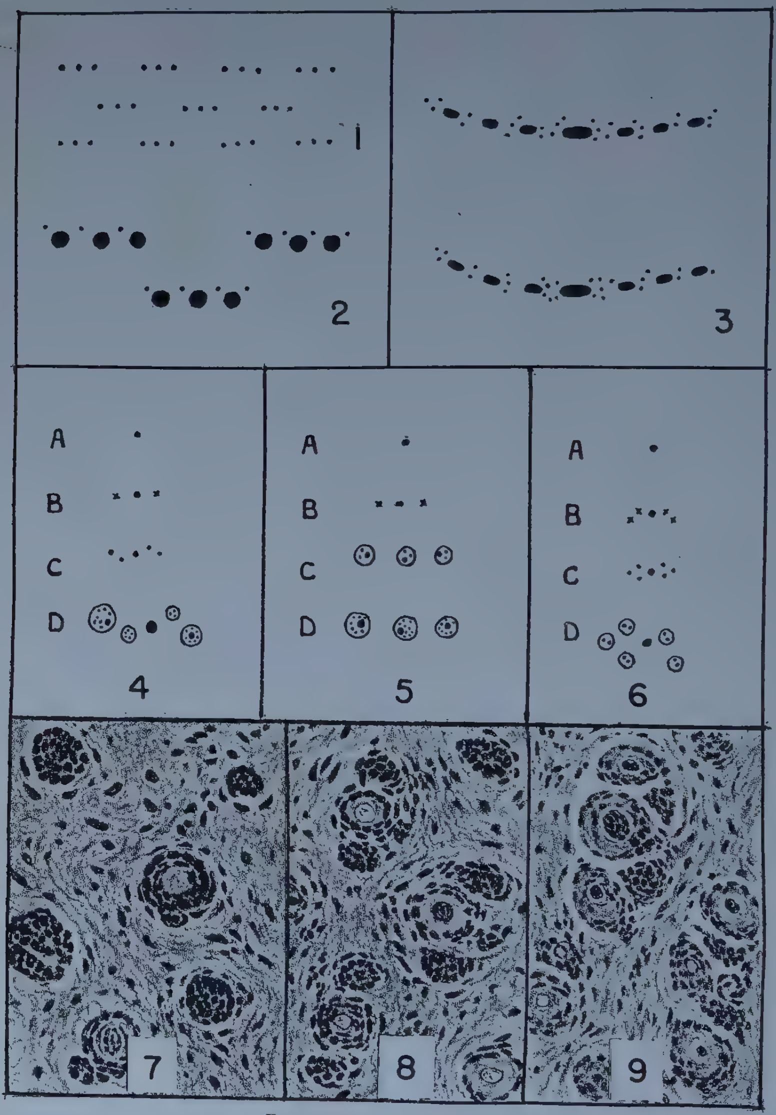
arrangement and a few intermingled coarse hairs (as in the back hairs of the cat, *Felis domesticus* in FIGURE 4D), and (8) hairs in irregularly scattered groups (as in the back hair of the raccoon, *Procyon cancrivorus*).

Dawson (1930) does not completely agree with DeMeijere's pattern in the guinea pig. She found variations in the pattern and no correlation between the size of hair and the arrangement of the hairs in each group. Histological study frequently shows follicle grouping which was not apparent to DeMeijere when he was examining only the skin surface, *e.g.*, in *Felis domesticus* (see Höfer, 1914). This indicates that analyses of the group pattern of hairs are needed in both common laboratory mammals and mammals in general.

In addition, DeMeijere analyzed the formation of the patterns by examining the skins of animals during their development (FIGURES 4–6). This phase of the problem has been extended to include a study of the ontogeny of the arrangement of hair follicles in sheep (Wildman, 1932, Galpin, 1935, and Duerden, 1939), in the cat (Höfer, 1914), in marsupials (Gibbs, 1938, Stoves, 1944b, and Hardy, 1946), in the mouse (Calef, 1900, Dry, 1926, and Gibbs, 1941) in the rat (Frazer, 1928), and in a number of mammals (Duerden, 1939). The terminology used by these authors in this problem is summarized in TABLE 1 (adapted from Wildman and Carter, 1939 and Carter, 1943).

Utilizing the terminology of Wildman and Carter, 1939, the following is a brief statement of the relation of the fiber generations. The first follicles to differentiate are the central trio follicles (FIGURE 7). If these follicles appear at two different times as in the opossum (Gibbs, 1938), then the follicles are called "primary X" and "primary Y." The essential point is that each of these primary follicles will be the central follicle of different hair groups. Later in development, other follicles of the hair group differentiate in relation to these central trio follicles. The trio is formed when two follicles are differentiated lateral to the primary follicles (FIGURE 8). The lateral follicles associated with primary X and primary Y are called respectively "primary x" and "primary y." If only one lateral follicle is formed adjacent to a primary follicle (X or Y), then a couplet follicle is formed. If no lateral follicles differentiate, a primary follicle (X or Y) is called a "solitary follicle." Later, another generation of follicles is differentiated—the secondary follicles. In the opossum (FIGURE 9), these secondary follicles are located between the central trio follicle and the lateral trio follicles. The ontogenetic studies of follicle arrangement have added confirmatory evidence to DeMeijere's basic concept that in mammals there is a universal and regular grouping of hair follicles (Hardy, 1946).

In general, the early differentiating follicles (central trio follicles) form the coarse overhair, while the late differentiating follicles (lateral trio follicles and secondary follicles) form the fine underhair. Lateral trio follicles sometimes at least produce overhair like that of the central follicles (*e.g.* in sheep) or intermediate types such as awns, which are classified by Danforth (1925a) as overhair. In *Ornithorhynchus anatinus* (Spencer and Sweet, 1899) and many marsupials (Gibbs, 1938, Bolliger and Hardy, 1945, Hardy, 1946), however, the lateral trio fibers are indistinguishable from those of secondary follicles, so it is difficult to place them in either the "overhair" or the "underhair" category.



FIGURES 1-9 (*see facing page*).

Spencer and Sweet (1899) claimed that, in monotremes, each group of follicles was differentiated by budding from the central follicle. This has not been described in marsupials or in eutherians, in which the follicles arise independently as epidermal downgrowths. Monotremes and marsupials have in common the fact that a follicle group typically contains a large central follicle with a sudoriferous gland, and two or more clusters of smaller lateral follicles (Spencer and Sweet, 1899, Gibbs, 1938, Hardy, 1946). This arrangement is also found in some eutherians, such as the cat (Höfer, 1914) and dog (Claushen, 1933). In the cat and a few other eutherians, the first-formed lateral follicles (primary x and y of the classification of Wildman and Carter, 1939) produce hairs intermediate in type between those of the central and the other lateral follicles. There are other eutherians in which the lateral primary x and y fibers are still more like the central primary X and Y fibers, as in the pig (Höfliger, 1931) and the sheep (Carter, 1943). Except in the rodents, there is always a sudoriferous gland opening into the central primary X or Y follicle (Hardy, unpublished data). Many animals, such as the pig and sheep, also have a sudoriferous gland opening into each primary x and y follicle, but others do not (Duerden, 1939). Some of the eutherians have only primary follicles in their skin, each with a sudoriferous gland. Findlay and Yang (1948) showed that this is the arrangement in cattle, and the same is probably true in horses and in human head hair (Hardy, unpublished observations).

Types of Hair

DeMeijere's analysis leads to the classification of hair types by Toldt (1910 and 1935) and by Danforth (1925a). Many details of the hair types in many species of animals and the variations of the structure of these types are described, illustrated, and bibliographically annotated by Toldt (1935) and Lochte (1938).

TYPES OF MAMMALIAN HAIR
(after Danforth, 1925*a*)

1. Hairs with specialized follicles containing erectile tissue. Large, stiff hairs that are preeminently sensory. They have been variously designated as feelers, whiskers,

---

FIGURES 1–9 (*see opposite page*).

FIGURE 1. The trio hair group pattern on the back and tail of the marmoset, *Midas rosalia* (after DeMeijere, 1896). All hairs have similar diameters.

FIGURE 2. The hair group pattern of more than 3 hairs with some fibers of greater diameter than other fibers on the back of the paca, *Coelogenys paca* (after DeMeijere, 1896).

FIGURE 3. The hair group pattern of more than 3 hairs with some fibers of greater diameter than other fibers on the back of the rodent, *Loncheres* (Echimys) *cristata* (after DeMeijere, 1896).

FIGURE 4. Ontogeny of a hair group on the back of the cat, *Felis domesticus*. A, from a newborn animal; B and C, from an older animal; and D, from an adult animal (after DeMeijere, 1896).

FIGURE 5. Ontogeny of a hair group on the back of the dog, *Canis familiaris*. A, from an embryo dog; B, from a newborn animal; C, from a young dog; and D, from an adult animal (after DeMeijere, 1896).

FIGURE 6. Ontogeny of a hair group on the back of the mouse, *Mus decumanus*. A, from a 7 cm. long animal; B, from a 9 cm. long animal; C, from a 12.5 cm. long animal; and D, from an adult animal.

(FIGURES 4, 5, and 6 illustrate that the follicle of the first hair to erupt (A) will be the follicle of the coarsest hair of the hair group in the adult. The type of hair group pattern in the adult (D) in each figure is noted in the text. The X in the diagrams marks the location of erupting follicles.)

FIGURE 7. The primary follicles X (the more differentiated follicles) and the primary follicles Y (the less differentiated follicles) in the transverse section of skin of a 12.5 cm. Australian opossum embryo (*Trichosurus vulpecula*). Follicles are scattered irregularly. (After Gibbs, 1938.)

FIGURE 8. Two new follicles (primary x or primary y) have become grouped with each previously differentiated follicle (primary X or primary Y) to form the typical trio arrangement. The trio would be either primary x, primary X, primary x or primary y, primary Y, primary y. Transverse section of skin of a 15.0 cm. Australian opossum embryo (*Trichosurus vulpecula*). (After Gibbs, 1938.)

FIGURE 9. Two secondary follicles have added to each trio group to form a 5 follicle group. The secondary follicles differentiate between the primary X (or Y) follicle and the primary x (or y) follicles. The five group would be either primary x, secondary follicle, primary X, secondary follicle, primary x or primary y, secondary follicle, primary Y, secondary follicle, primary y. Transverse section of skin from 20.0 cm. Australian opossum embryo (*Trichosurus vulpecula*). Note presence of a dermal capsule surrounding each 5 follicle group. (After Gibbs, 1938.)

(In FIGURES 7, 8, and 9, the terminology of Wildman and Carter (1939), noted in the text, is used.)

TABLE 1

FIBER-FOLLICLE TERMINOLOGY IN THE MAMMALIA*

Wildman and Carter (1939)		*Duerden (1939) (sheep)*	*Hardy (1946) (marsupials)*	*Gibbs (1938) (opossum)*	*Galpin (1935) (sheep)*	*Wildman (1932) (sheep)*	*Toldt (1911) (various animals) and Höfer (1914) (cat)*
Primary follicles		trio follicles					
(a) Central trio follicles	primary X primary Y	central trio follicles	central follicle	primary follicles secondary follicles	X follicle Y follicle	primary follicle secondary follicle	Mittelhaar follicle
(b) Lateral trio follicles	primary x primary y	lateral trio follicles	large lateral follicle	tertiary follicles	x follicle y follicle	lateral trio follicle	Stammhaar follicles
(c) Couplet follicles	primary X + x primary Y + y						
(d) Solitary follicles	primary X or primary Y						
Secondary follicles		post-trio follicles	lateral follicles	quaternary follicles			Beihaar follicles

* Adopted from Wildman and Carter (1939).

sensory hairs, sinus hairs, tactile hairs, vibrissae, *etc.* They occur in all mammals except man, and are grouped by Botezat (1914) (Pocock, 1914) essentially as follows:

(1). Active tactile hairs—under voluntary control.

(2) Passive tactile hairs—not under voluntary control.

- (*a*) Follicles characterized by a circular sinus.
- (*b*) Follicles without a circular sinus.

2. Hairs with follicles not containing erectile tissue. The remaining types of hair, most of which are more or less defensive or protective in function. In many cases, the follicles have a good nerve supply, endowing the hair with a passive sensory function as well. These hairs are grouped here according to their size and rigidity.

(1). Coarser, more or less stiffened "overhair," guard hair, top hair.

- (*a*) Spines. Greatly enlarged and often modified defensive hairs, quills.
- (*b*) Bristles. Firm, usually subulate, deeply pigmented, and generally scattered hairs. "Transitional hairs" (Botezat, 1914), "Leithaare" (Toldt, 1910), "protective hair," "primary hair," "overhair." This group also includes mane hairs.
- (*c*) Awns. Hairs with a firm, generally mucronate tip but weaker and softer near the base. "Grannenhaare" (Toldt, 1910), "overhair," "protective hair."

(2). Fine, uniformly soft "underhair," "ground hair," "underwool."

- (*a*) Wool. Long, soft, usually curly hair.
- (*b*) Fur. Thick, fine, relatively short hair—"underhair," "wool hair."
- (*c*) Vellus. Finest and shortest hair—"down," "wool," "fuzz," "lanugo." (Danforth, 1939).

The following comments supplement the above classification. The guard hairs are listed in a series from greater to lesser rigidity (in order: spines, bristles, and awns). There are many intergrade hairs between the typical bristle and the typical awn and between the typical awn and the typical fur hair (FIGURES 10, 11, and 12).

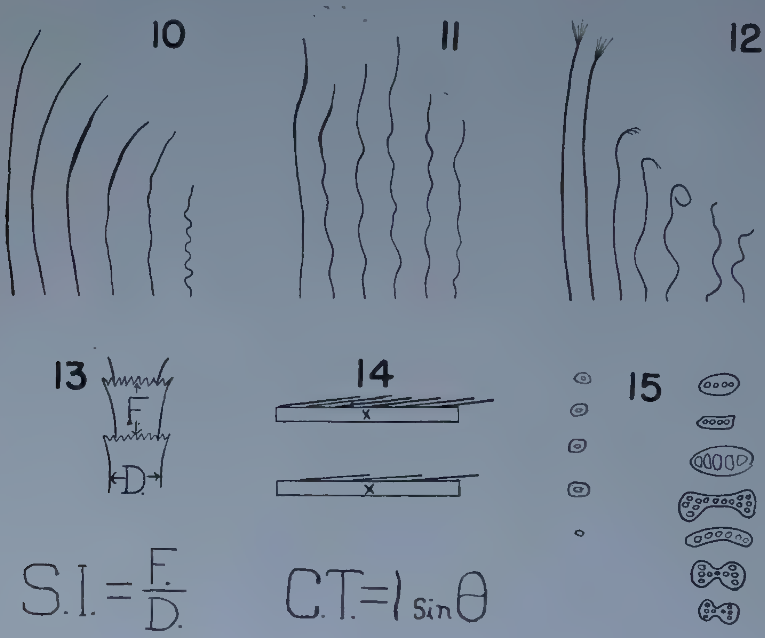
The tactile hairs have a rich nerve supply, while the roots of some are encircled by large circular sinuses containing erectile tissue. When the pressure in the circular sinus is increased the hair becomes a more efficient pressure receptor. The overhairs have a definite nerve supply, while the underhairs have no direct nerve supply. As a general but not absolute rule, the coarser hairs appear ontogenetically earlier than the finer hairs (Gibbs, 1938, Danforth, 1925*a*, Duerden, 1937 (reported by Wildman, 1937), Höfer, 1914, and Spencer and Sweet, 1899).

The contour, diameter, and shape of a hair fiber changes from its root to its tip (Note awns, FIGURES 16–18). The cross-sectional outline of hairs may vary from the thick rounded porcupine quill to the eccentric flattened hairs of seals. The former serves a protective function, while the latter is adapted to hug to the skin so as not to hinder aquatic locomotion. Many details of the anatomy of hair form are noted by Stoves (1942 and 1944a), Toldt (1935), and Lochte (1938).

It is possible for a hair follicle to differentiate one type of hair at one stage and another type at another stage. The follicle of a bristle (kemp) of the Merino lamb may become the follicle of wool in the adult sheep (Duerden, 1937, reported by Wildman, 1937). A fine lanugo hair of the human fetus is associated with a follicle which will later be the follicle of a coarser hair.

The theories of hair curling are reviewed by Herre and Wigger (1939). The curling of hair in primitive sheep is independent of the arrangement of hair, existence of hair whorls, or the cross section of the hair (Pfeifer, 1929).

Wildman (1932) suggests that the shape of the follicle, especially the curve in its basal portion, is a possible factor in hair curling. Reversal of the spiral in some wool fibers may be explained according to Wildman as due to a shift in the growing point of the follicle and inner root sheath. Spiral reversal occurs in human hair (Danforth, 1926). Pfeifer (1929) doubts that curling is determined by a curve of the follicle alone and suggests that Tänzer's (1926) contention that the follicle must be saber-shaped is important. Waving of all compact wools is due at least in part to the flattening of the primary spiral and to the unequal lateral growth of the fiber (Duerden, 1927). The curling of hair in karakul sheep fetuses may be associated with the differences in the rates of growth in the various skin layers (Herre and Wigger, 1939).



FIGURES 10–15.

FIGURE 10. The hair of the fox, *Canis vulpes* (after Toldt, 1935), illustrating intergrade hairs. From the left to the right, Toldt named the fibers Leithaar (bristles), Leit-Grannenhaar, thick Grannenhaar (awns), thin Grannenhaar, Grannen-Wollhaar, and Wollhaar (fur).

FIGURE 11. The hair of the chinchilla, *Chinchilla laniger* (after Toldt, 1935) illustrating an animal hair coat with hairs of approximately the same length. The 2 hairs on the left are awns, and the rest, either intergrade hairs or fur hairs.

FIGURE 12. The hair of the wild pig, *Sus scrofa* (after Toldt, 1935) illustrating bristles on the left and underhair on the right with some intergrade hairs between them. Note the brushlike distal ends of the bristles.

FIGURE 13. The scale index (S. I.), according to Hausman (1930), is equal to the ratio of the free proximo-distal length of a scale (F) to the diameter of the hair shaft (D).

FIGURE 14. The thickness of the cuticle (C. T.), according to Rudall (1941), is equal to the length of a cuticular scale (l) times the sine of angle (sin Θ) the scale makes with the cortex (X).

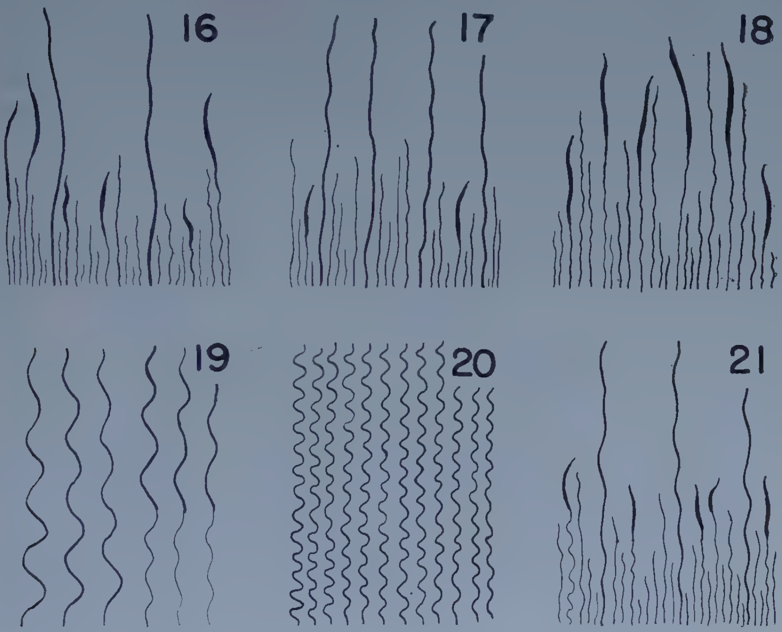
FIGURE 15. Cross sections of several regions of a fur hair (left) and an awn (right) of the rabbit (after Toldt, 1935). The sections, at the top of the figure, are from the base of the hair and, at the bottom of the figure, from the tip of the hair. Illustrates general uniformity of the diameters of the fur hair and differences in diameters and contour of awn hairs throughout their lengths.

The factors responsible for curling and crimping of hair are as yet not completely known.

Structural Components of Hair

The cuticle, cortex, and medulla are the three structural components in hair. They will be discussed in order.

Cuticle. The cuticle consists of thin, unpigmented, transparent overlapping scales, whose free margins are oriented toward the tip of the hair (FIGURE 22). Within the follicle, the free margins of the hair cuticular scales interlock with the inner root sheath cuticular scales, which are oriented in the opposite direction toward the papilla. This interlocking of scales helps to secure the hair in place (Danforth, 1925*a*). The cuticle functions as a capsule containing the longitudinally splitable cortex (Rudall, 1941). This explains why the cortex of a hair frays at its severed end. In addition, the cuticle, with its oily layer, prevents the transfer of water (Rudall, 1941).



FIGURES 16–21.

FIGURE 16. Diagram of the fiber components of coat of a generalized non-wooled animal (after Duerden, 1929). Note presence of bristles (coarse fibers), awns (fibers with fine basal segments and coarse distal segments) and fur fibers (fine fibers).

FIGURE 17. Diagram of the fibers of the wild sheep (after Duerden, 1929). Note the presence of bristles (kemp), awns (heterotypes), and wool.

FIGURE 18. Diagram of the fibers of British mountain breeds (after Duerden, 1929). The fibers are mainly awns and wool. Few bristles are present.

FIGURE 19. Diagram of the fibers of the British luster breeds (after Duerden, 1929). Fibers on the left are wool fibers which are coarser than the wool fibers of wild sheep. The fibers on the right are modified awns with fine proximal segments and slightly coarse distal segments. All fibers are elongated and spiraled.

FIGURE 20. Diagram of fibers of adult Merino sheep (after Duerden, 1929). All fibers are wool. Note uniformity of all fibers as to size, length, and waviness. These wool fibers are coarser than wool fibers from wild sheep. Unlike the fibers of other breeds, the fibers of the adult Merino sheep grow from persistent germs and do not shed.

FIGURE 21. Diagram of the fibers of the Merino lamb. Note the presence of bristles (kemp), awns (heterotypes), and wool. During later development, the bristles are shed and the distal coarse segments of the awns are lost. The adult coat is formed by the persistent growth of the wool fibers of the lamb, by the replacement of wool in the follicles of the shed kemp, and by the persistence of the growth of the proximal segments of the awns.

The cuticular scales vary in thickness from 0.5 to 3 micra (Frölich, Spötel, and Tänzer, 1929). Since the scales overlap, the number of overlapping scales at any point on the hair surface determines the thickness of the cuticle. The cuticular thickness may be expressed as being equal to the length of the scales times the sine of the angle the scale makes with the cortical surface (FIGURE 14, Rudall, 1941).

The cuticular scales may be classified into two types: coronal scales and imbricate scales (Hausman, 1930). A coronal scale completely encircles the hair shaft. They are subdivided according to the contour of the free margins as: simple, serrate, or dentate (FIGURE 23). Müller (1939) contends that a coronal scale is in reality several scales whose lateral edges are fused. For example, a dentate coronal scale with 5 processes in its free border is the fused product of 5 elongated pointed scales.

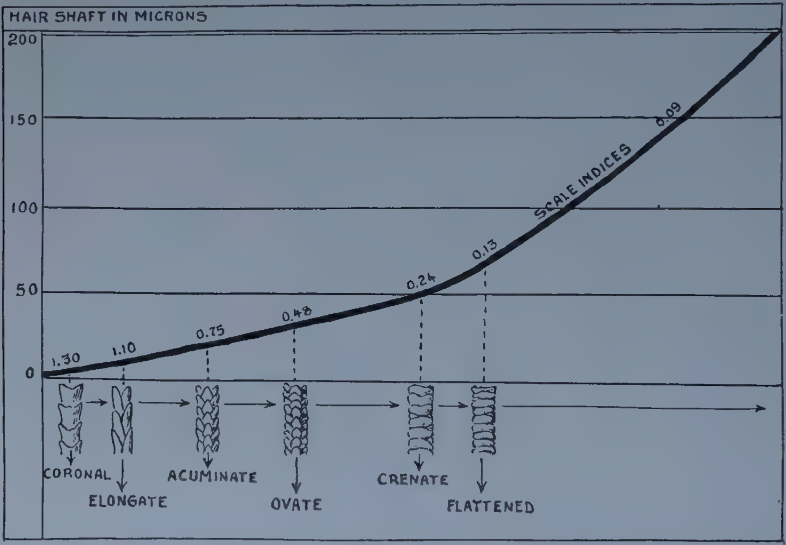


FIGURE 22. Graph illustrating the relation of the diameter of the hair to the types of cuticular scales. The finest hairs (with small diameters) have a high-scale index and coronal scales. The coarsest hairs (with large diameters) have a low-scale index and flattened scales. Diameters of hair shafts are plotted on the ordinate. General regions of the occurrence of scale forms are shown along the abscissa, the average scale indices along the curve. The figures of the scale types beneath the graph are not drawn to scale. (After Hausman, 1930.)

An imbricate scale does not completely surround the hair shaft. They are classified as ovate, acuminate, elongate, crenate, and flattened (FIGURE 22, Hausman, 1930).

Hausman (1930) devised a scale index to express the relation between the diameter of the hair shaft and the free proximo-distal dimension of the scales (FIGURE 13). The free proximo-distal dimension is actually a means of expressing the type of scale. For example, coronal scales have a large proximo-distal dimension, while crenate scales have a small dimension (FIGURE 22). An analysis of the scale indices indicates that a relation exists between the types of scales and the shaft diameters. In general, the finest hairs have large scale indices and coronal scales, while the coarsest hairs have small scale indices and crenate or flattened scales. On the basis of the above, it is concluded that the types of cuticular scales present on hair are related not to the taxonomic status of the animal possessing the hair but rather to the diameter of the hair shaft (Hausman, 1930). In hairs with both thick and thin segments, the thick segments have the scale types of large diameter hairs while the thin segments have the scale types of small diameter hairs.

A coarse guard hair has scales with free lips that are closely applied to the cortex and are scarcely raised. As a result, these hairs have a high luster (due to unbroken reflection of light from the hair surface) and do not interlock with other hairs. A fine underhair has scales with lips that have raised margins. As a result, these hairs are dull (due to broken reflection of light) and interlock with other fine hairs. Thus, mohair has a high luster but makes poor felt, while wool is dull but makes good textiles.

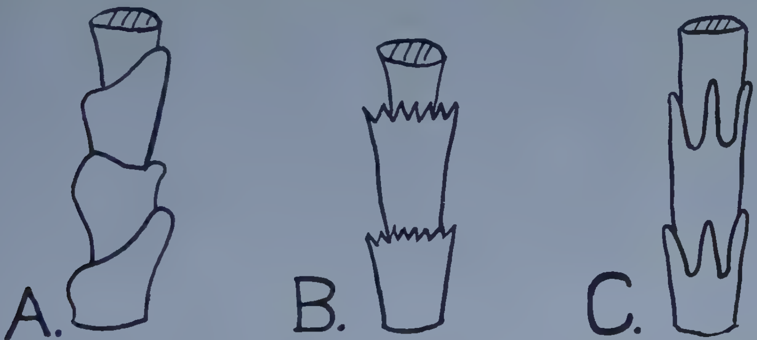


FIGURE 23. Figures illustrating the types of coronal cuticular scales. A. simple scales, B. serrate scales, C. dentate scales, (after Hausman, 1930). Note raised margins on the free lips of scales.

Many details of the cuticle in many species of animals are presented and illustrated by Lochte (1938).

Cortex. The cortex usually forms the main bulk of a hair. It is a column of fusiform keratinized cells which are coalesced into a rigid, almost homogeneous, hyaline mass (Hausman, 1932). Damaged hairs tend to split lengthwise because the elongated cortical cells are oriented longitudinally. The cortex has such a low refractive index—due to the degree of cornification—that, in the absence of pigment, it is translucent. Since cortical scales have not been analyzed in such detail as cuticular scales, no statement can be made of a relation between cortical scale morphology and hair size. The form and distribution of the pigment in the cortex and the medulla is noted by Lochte (1938), Toldt (1935), and Hausman (1930).

Hausman (1932 and 1944) analyzed the cortical air spaces known as cortical fusi—cortical in location and fusiform in shape—air vacuoles, air chambers, air vesicles, or vacuoles. As the irregular-shaped cortical cells located in the bulb rise to the follicular mouth, they carry between them cavities filled with tissue fluid. As the hair shaft dries out, the cavities lose the fluid, and air may fill the resulting spaces—the fusi. The shape of the fusi vary. They are largest, most numerous, and most prominent near the base of the hair, and they are filiform and thin or lost in the distal segments of the hair. Seldom do they persist to the tip of a hair. As a rule, they are visible only under a microscope. Hausman implies that there is a relation between fusi and hair size. Presumably, the coarser a hair segment is, the more numerous the fusi.

Ringed hair results when the fusi appear in masses at regular intervals in the shaft. Fractured fusi result when hairs are damaged sufficiently to separate the cortical cells enough to allow air to collect between them. Fusi can be distinguished from pigment granules, for they are fusiform, whereas pigment granules have blunt ends.

The presence of a thin membrane located between the cuticle and the cortex has been assumed by Lehmann (1944). Observations of pigment granules, cell nuclei, and submicroscopic fibrils are presented by Mercer (1942), Hausman (1930), and others.

Medulla. The medulla (pith), when present, is composed of shrunken and variably shaped cornified remnants of epithelial cells connected by a filamentous network. In contrast to the cortex, the medulla is less dense and has fewer and larger cells, which are more loosely held together. In the medulla are air cells or chambers, which are filled by a gas, probably air. These air cells may be intracellular (deer) or intercellular (dog, weasel, and rat) (Lochte, 1938). The intercellular air cells are classifiable according to their coarseness and arrangement (Lochte, 1934 and 1938).

Medullas are classified by Hausman (1930) as follows: absence of medulla, discontinuous medulla (air cells separate), intermediate medulla (several separate air cells of the discontinuous type arranged into regular groups), continuous medulla (air cells arranged to form a column), and fragmental medulla (air cells arranged into irregular groups). These types are illustrated in FIGURE 24 and are arranged in the order of the sizes of hairs in which they are located. In the finest hairs (underfur), the medulla is either absent or of the discontinuous type. In the coarsest hairs, the medulla is either of the continuous or the fragmental type (FIGURE 24). If a hair varies in thickness, its medulla will vary. For example, in the awns of sheep, the distal thickened segment has a medulla, while the fine proximal segment may have no medulla. The arrangement of the medullary air cells is related not to the taxonomic group of the animal possessing the hair nor the age of the hair, but rather to the diameter of the hair shaft (FIGURE 24) (Hausman, 1930; Wynkoop, 1929; and Smith, 1933). The sheens and colors of hairs are largely determined by the light reflected from the medulla (Hausman, 1944).

Although the cortex forms the bulk of the shaft in most hairs, the medulla assumes large proportions in some hairs. In rabbit hair (FIGURE 15), the medulla is composed of large air cells separated by little more than a framework of cortex (Stoves, 1944*a*).

The significance of the cuticle, cortex, and medulla in the commercial aspects of fur is presented by Bachrach (1946). Although many of the details of the structural elements of hair cannot be definitely utilized to identify an animal species (Hausman, 1944), it is possible that some morphological features of hair can be used (Williams, 1938).

Some chemical and physical aspects of the morphological elements of hair have been analyzed. Not only do the cuticle, cortex, and medulla exhibit different chemical and physical properties, but various segments of these structural elements may have different chemical and physical properties (Rudall, 1944; Stoves, 1943*b*, 1945; Lustig, Kondritzer, and Moore, 1945; Leblond, 1951; and Giroud and Leblond, 1951).

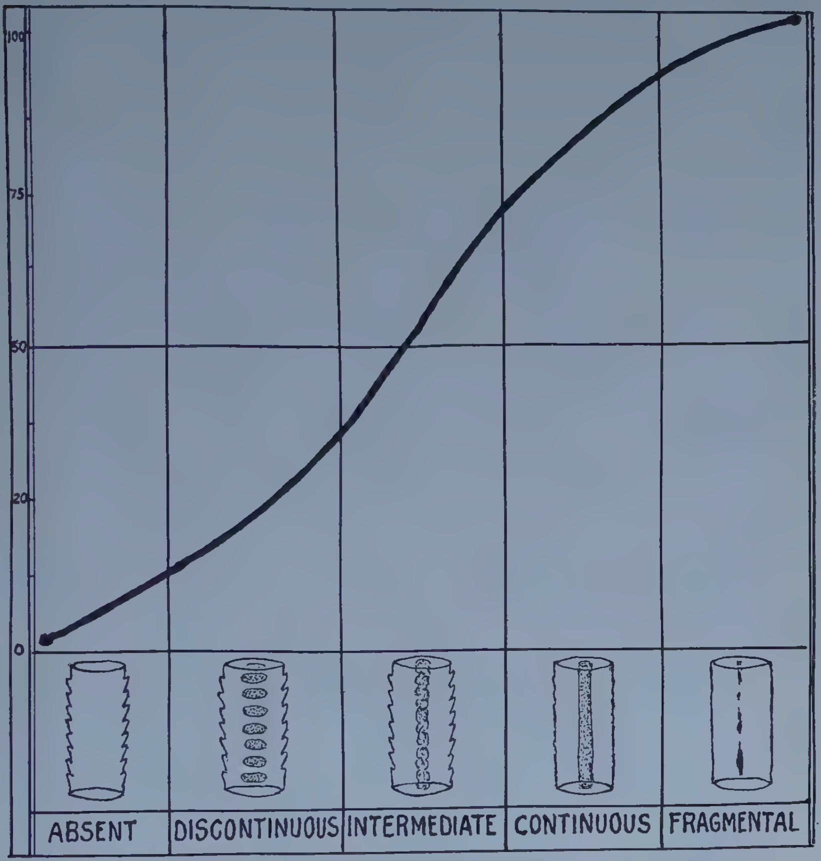


FIGURE 24. Graph illustrating the relation of the diameter of the hair to the types of medullas. The finest hairs have no medulla, and the coarsest hairs have a fragmental medulla. Diameters in micra of the hair shafts are plotted on the ordinate. The figures of the types of medullas beneath the graph are not drawn to scale. (After Hausman, 1930.)

Some Phylogenetic Aspects of Hair

The relation of hair to the epidermal structures in non-mammalian animals has been discussed by many authors and has been summarized by

Botezat (1913 and 1914), Danforth (1925b), and Matkeiev (1932). No direct relation between hair and non-mammalian epidermal elements has been established. Hair is most probably an analog to these structures. Danforth (1925b) and others conclude that hair is probably a *de novo* morphological entity in mammals.

Broili (1927) reports that he identified hair and hair follicles in a fossil aquatic reptile, *Rhamphorynchus*. This animal is a specialized reptile, removed from those reptiles in the evolutionary line to mammals. If established, this observation would alter the concept that only mammals produce hair.

The phylogeny of hair in related groups of animals has not been analyzed extensively. Because of the economic importance of wool, several studies of the hair types in the coat of a number of breeds of sheep have been made. One significant aspect of these studies is that they illustrate how the hair coat may vary in closely related forms.

This statement is adapted primarily from Duerden (1927 and 1929). The generalized wild animal hair coat consists of an overcoat of bristles and awns and an undercoat of fur (FIGURE 16). In the wild sheep and the black-headed Persian sheep, the hair coat is similar to that of the wild animal. These sheep have an overcoat of bristles (called kemp) and awns (called heterotypes) and an undercoat of wool (FIGURE 17). The British mountain breeds have a hair coat consisting of awns and wool (FIGURE 18). In these breeds, kemp formation is negligible. The coat of the British luster breeds have evolved in another direction. The awns retain their fine proximal segments. Their distal segments are still thicker than the proximal segments, but are thinner than the distal segments of the awns of primitive sheep. The wool undercoat fibers have thickened. Both fiber types are elongated and spiraled (FIGURE 19). The adult Merino sheep, the most efficient wool-producing sheep, has a coat consisting of elongated, regularly crimped fibers of uniform diameters and lengths (FIGURE 20). An analysis of the coat of the Merino lamb is essential for the identification of the types of fibers that form the coat of the adult sheep. The Merino lamb coat has bristle, awn, and wool fibers (FIGURE 21). During ontogeny, the bristles are shed and then replaced by wool fibers. The awn fibers lose their distal thickened segments, but the thin proximal segments persist. The wool fibers are retained, but are coarser than the wool of primitive sheep. Hence, the adult Merino sheep coat consists of wool fibers differentiating from follicles which produced kemp in the lamb, of awns deprived of their distal segments, and of wool fibers differentiating from follicles which produced wool in the lamb. A major difference between the Merino sheep and other sheep is in the nature of the hair follicles. Whereas the coat of other breeds is shed periodically and then new hairs differentiate from the follicles, the fibers of the adult Merino sheep grow from persistent germs and are not shed.

In the evolution of the sheep coat from primitive wild sheep to the various domestic breeds, several changes have occurred. As summarized by Duerden (1927), the domestic wooled sheep has evolved in the direction of the loss of the protective coat both of bristles (kemp) and awns (heterotypes), the increase in length, density, and uniformity of the fibers, and the tendency of the retained bristles to become finer but still capable of being shed. In addition, the Merino sheep has developed persistently growing hair follicles.

Important implications of the evolution of the sheep coat are that the types of hair in the hair coat may differ (1) in closely related animals and (2) at various stages of ontogeny with the same animal. Hence, data derived from a study of the coat of one animal species may not always apply to another animal species.

Bibliography

American Society for Testing Materials. 1948. Standards on textile materials. By the Society for Testing Materials. 560 pp. Philadelphia.

Bachrach, M. 1946. Fur. 672 pp. Prentice-Hall. New York.

Bolliger, A. & M. H. Hardy. 1945. The sternal integument of *Trichosurus vulpecula*. Proc. Roy. Soc. New South Wales **78:** 122–133.

Bonnet, R. 1892. Ueber Hypotrichosis congenita universalis. Anat. Hefte **1:** 233–273.

Botezat, E. 1913. Ueber die Phylogenie der Säugetierhaare. Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte. **85:** 696–698.

1914. Phylogenese des Haares der Säugetiere. Anat. Anz. **47:** 1–44.

Broili, F. 1927. Ein Rhamphorhynchus mit Spuren von Haarbedeckung. Sitzungsber. Math.-Naturw. Abt. Bayerische Akad. Wiss. München 49–68.

Calef, A. 1900. Studio isologico e morfologica di un' appendice epitaliale del pelod nella pelle del Mus dectmanus var. albina e del Sus scrofa. Anat. Anz. **17:** 509–517.

Carter, H. B. 1943. Studies in the biology of the skin and fleece of sheep. Council for Scientific and Industrial Research, Commonwealth of Australia. Bulletin No. 164: 1–59.

Claushen, A. 1933. Mikroskopische Untersuchungen über die Epidermatgebilde am Rumpfe des Hundes mit besonder Berücksichtigung der Schweissdrüsen. Anat. Anz. **77:** 81–97.

Danforth, C. 1925*a*. Hair, with special reference to hypertrichosis. American Medical Association. Chicago, Illinois; Arch. of Derm. and Syph. **11:** 494–508, 637–653, 804–821.

Danforth, C. 1925*b*. Hair in its relation to questions of homology and phylogeny. Am. J. Anat. **36:** 47–68.

Danforth, C. 1926. The hair. Nat. Hist. **26:** 75–79.

Danforth, C. 1939. Physiology of human hair. Physiol. Rev. **19:** 94–111.

Dawson, H. L. 1930. A study of hair growth in the guinea pig (*Cavia cobaya*). Amer. J. Anat. **45:** 461–484.

DeMeijere, J. C. H. 1894. Über die Haare der Säugethiere besonders über ihre Anordung. Gegenbaur's Morphol. Jahrb. **21:** 312–424.

Dry, F. W. 1926. The coat of the mouse (*Mus musculus*). J. Genetics **16:** 287–340.

Duerden, J. E. 1927. Evolution in the fleece of sheep. S. Afri. J. Sci. **24:** 388–415.

Duerden, J. E. 1929. The zoology of the fleece of sheep. S. Afri. J. Sci. **26:** 459–469.

Duerden, J. E. 1939. The arrangement of fibre follicles in some mammals with special reference to the Ovidae. Trans. Roy. Soc. Edinburgh **59:** 763–771.

Findlay, J. D. & S. H. Yang. 1948. Capillary distribution in cow skin. Nature, Lond. **161:** 1012–1013.

Frazer, D. A. 1928. The development of the skin of the back of the albino rat until the eruption of the first hairs. Anat. Rec. **38:** 203–224.

Frölich, G., W. Spöttel, & E. Tänzer. 1929. Wollkunde: bildung und eigenschaften der wolle. 419 pp. J. Springer. Berlin.

Galpin, N. 1935. The prenatal development of the coat of the New Zealand Romney lamb. J. Agri. Sc. **25:** 344–360.

Gibbs, H. F. 1938. A study of the development of the skin and hair of the australian opossum Trichosurus vulpecula. Proc. Zool. Soc. London **108:** 611–648.

Gibbs, H. F. 1941. A study of the post-natal development of the skin and hair of the mouse. Anat. Rec. **80:** 61–82.

GIROUD, A. & C. P. LEBLOND. 1951. The keratinization of epidermis and its derivatives, especially the hair, as shown by X-ray diffraction and histochemical studies. Ann. N. Y. Acad. Sci. **53** (3): 613.

HARDY, M. 1946. The group arrangement of hair follicles in the mammalian skin. Notes on follicle group arrangement in 13 australian marsupials. Proc. Roy. Soc. Queensland **58:** 125–148.

HAUSMAN, L. A. 1930. Recent studies of hair structure relationships. Sc. Month. **30:** 258–277.

HAUSMAN, L. A. 1932. The cortical fusi in mammalian hair shafts. Amer. Nat. **66:** 461–470.

HAUSMAN, L. A. 1934. Histological variability of human hair. Amer. J. Phys. Anthrop. **18:** 415–429.

HAUSMAN, L. A. 1944. Applied microscopy of hair. Sc. Month. **59:** 195–202.

HERRE, W. & H. WIGGER. 1939. Die Lockenbildung der Säugetiere. Kühn-Archiv. **52:** 233–254.

HÖFER, H. 1914. Das Haar der Katze, seine Gruppenstellung und die Entwicklung der Beihaare. Arch. f. Mik. Anat. **85:** 220–278.

HÖFLIGER, H. 1931. Haarkleid und Haut des Wildschweines. VII. Beitrag zur Anatomie von Sus scrofa L. und zum Domestikations-problem. Z. Ges. Anat. 1. Z. Anat. Entw. Gesch. **96:** 551–623.

LEBLOND, C. P. 1951. Histological structure of hair, with a brief comparison to other epidermal appendages and epidermis itself. Ann. N. Y. Acad. Sci. **53** (3): 464.

LEHMANN, E. 1944. Neue Reactionen der Tierischen Faser. Kolloid-Zeitschrift. **108:** 6–10.

LOCHTE, T. 1934. Untersuchungen über die Unterscheidungsmerkmale der Deckhaare der Haustiere. Deutche Zeitschr. Ges. Genchtl. Med. **23:** 267–280.

LOCHTE, T. 1938. Atlas der Menschlichen und Tierschen Haare. 306 pp. Paul Schops. Leipsig.

LUSTIG, B., A. KONDRITZER, & D. MOORE. 1945. Fractionation of hair, chemical and physical properties of hair fractions. Arch. Biochem. **8:** 57–66.

MATKEIEV, B. S. 1932. Zur theorie der Rekapitulation. Über die Evolution der Schuppen, Federn und Haare auf dem Wege embryonaler Veränderungen. Zool. Jahrb. Abt. Anat. u. Ontog. **55:** 555–602.

MERCER, F. H. 1942. Some experiments on the structure and behavior of the cortical cells of wool fibers. J. Council Sc. Ind. Research **15:** 221–227.

MÜLLER, C. 1939. Ueber den Bau der koronalen Schüppchen des Säugetierhaares. Zool. Anz. **126:** 97–107.

PAX, F. & W. ARNDT. 1929–1938. Die Rohstoffe des Tierreichs. 2235 pp. Gebrüder Borntraeger, Berlin. Sections in this reference are: FRÖLICH, G., W. SPÖTTEL, & E. TÄNZER. 1932. Haare und Borsten der Haussäugetiere. **1:** 995–1221; MEISE, W. 1933. Menschenhaar. **1:** 1312–1363; SCHLOTT, M., E. BRASS, W. STICHEL, & E. KLUMPP. 1930. Pelze. **1:** 405–567; SCHLOTT, M. 1933. Haare und Borsten von Wildsäugern. **1:** 1222–1311.

PFEIFER, E. 1929. Untersuchungen über das histologisch bedingte Zustandekommen der Lockung, mit besonderer Berücksichtigung des Karakullammes. Biol. Generalis. **5:** 239–264.

PINKUS, F. 1927. Die normale Anatomy der Haut. In: Handbuch der Haut- und Geschlechtskrankheiten. **1:** 1–378. J. Springer, Berlin.

POCOCK, R. 1914. On the facial vibrissae of mammalia. Proc. Zool. Soc. Lond. **40:** 889–912.

RUDALL, K. M. 1941. The structure of the hair cuticle. Proc. Leeds Philos. Soc. **4:** 13–18.

SMITH, H. H. 1933. The relationships of the medulla are cuticular scales of the hair shafts of the Soricidae. J. Morph. **55:** 137–149.

SMITH, S. & J. GLAISTER. 1939. Recent Advances in Forensic Medicine. 264 pp. P. Blakiston's Sons and Co. Phila.

SPENCER, B. & G. SWEET. 1899. The structure and development of the hairs of monotremes and marsupials. Part 1. Monotremes. Quart. J. Micro. Sci. (N. S.) **41:** 549–588.

STOVES, J. L. 1942. The histology of mammalian hair. Analyst **67:** 385–387.

STOVES, J. L. 1943*a*. The biological significance of mammalian hair. Proc. Leeds Phil. and Literary Soc. **4:** 84–86.

STOVES, J. L. 1943*b*. Structure of keratin fibres. Nature **151:** 304–305.

STOVES, J. L. 1944*a*. The appearance in the cross sections of the hairs of some carnivores and rodents. Proc. Roy. Soc. Edinburgh **62B:** 99–104.

Stoves, J. L. 1944*b*. A note on the hair of the South American opossum (*Didelphis caranophaga*). Proc. Leeds Philos. Soc. **4:** 182–183.

Stoves, J. L. 1945. Histochemical studies of keratin fibres. Proc. Roy. Soc. Edinburgh. **62:** 132–136.

Tänzer, E. 1926. Haut und Haar beim Karakul im rassenanalytischem Vergleich. Halle a.d.S. (cited by: Pfeifer, 1929).

Toldt, K., Jr. 1910. Über eine beachtenswerte Haarsorte und über das Haarformensystem der Säugetiere. Annalen d. K. K. Naturhistorischen Hofmuseums in Wien. **24:** 195–265.

Toldt, K., Jr. 1912. Beträge zur Kenntnis der Behaarung der Säugetiere. Zool. Jahrb. Abt. Syst. **33:** 9–86.

Toldt, K., Jr. 1935. Aufbau und natürliche Färbung des Haarkleides der Wildsäugetiere. 291 pp. Deutsche Gesellschaft fur Kleintier- und Pelztierzucht. Leipsig.

Trotter, M. 1932. The hair. Special Cytology (Cowdry, E. ed.) **1:** 39–65. P. Hoeber. New York.

VonBergen, W. & W. Krauss. 1942. Textile fiber atlas. Amer. Wool Handbook Co. New York.

Wildman, A. B. 1932. Coat and fibre development of some british sheep. Proc. Zool. Soc. London **1:** 257–285.

Wildman, A. B. 1937. Non-specificity of the trio follicles in the Merino. Nature, Lond. **140:** 891–2.

Wildman, A. B. 1940. Animal fibres of industrial importance: their origin and identification. 28 pp. Wool Industries Research Assoc. Torridon, Headingley, Leeds.

Wildman, A. B. & H. Carter. 1939. Fibre-follicle terminology in the mammalia. Nature **144:** 783–784.

Williams, C. 1938. Aids to the identification of mole and shrew hairs with general comments on hair structure and hair determination. J. Wildlife Management **2:** 239–250.

Wynkoop, E. M. 1929. A study of the age correlations of the cuticular scales, medullae and shaft diameters of human head-hair. Am. J. Phys. Anthrop. **13:** 177–188.

Discussion of the Paper

Doctor M. H. Hardy (*McMaster Laboratory, Glebe, N. S. W., Australia*): I am glad Dr. Noback mentioned sheep, because the study of the arrangement of follicles in groups on these animals has disclosed some important principles. Terentjeva,[1] Duerden,[2] and Carter[3] showed that de Meijere's trio group is the basic unit in the follicle population of sheep. The trio (primary) follicles develop first and have accessory structures (sudoriferous gland, arrector pili muscle) which are absent from the later developing (secondary) follicles of the group.[3] In the young lamb, it is the primary follicles which produce the coarse, and frequently medullated, kemp fibers and the secondary follicles which produce the fine and usually non-medullated wool fibers. These correspond respectively to the 'overhair' and 'underhair' in Danforth's classification. The primary follicles may produce kemp in the lamb and wool in the adult sheep, as Dr. Noback has mentioned.

The size of the follicle groups, *i.e.*, the number of secondary follicles to each trio of primary follicles, varies greatly between breeds[4] and individuals[5] and also between body regions.[6] The breeds and, to some extent, individuals with the largest group size have also the greatest number of fibers to the square inch and the greatest uniformity of fiber thickness and length.[5] In the midside region, at least, the potential group size (including secondary follicle rudiments in the young lamb) is strongly inherited, but the actual group size (number of active follicles) in the mature animal varies according to the food intake in the first year of life.[7] Thus, it is possible to alter the group size experimentally. Varying the food intake in the second and third year of life had no marked effect on group size.[8,9]

It seems that many properties of the coat of the sheep depend on the inherited follicle group pattern and the modifications of this superimposed by the environment. Perhaps the same principles apply to other mammals.

1. TERENTJEVA, A. A. 1939. Pre-natal development of the coat of some fine-wooled breeds of sheep. C. R. Acad. Sci. U.R.S.S. (N.S.) **25:** 557.
2. DUERDEN, J. E. 1939. The arrangement of fibre follicles in some mammals, with special reference to the Ovidae. Trans. Roy. Soc. Edin. **59:** 763.
3. CARTER, H. B. 1943. Studies in the biology of the skin and fleece of sheep. 1. The development and general histology of the follicle group in the skin of the Merino. Coun. Sci. Ind. Res. (Aust.) Bull. 164: 7.
4. CARTER, H. B. & P. DAVIDSON. Unpublished data.
5. CARTER, H. B. 1942. "Density" and some related characters of the fleece in the Australian Merino. J. Coun. Sci. Ind. Res. (Aust.) **15:** 217.
6. CARTER, H. B. & M. H. HARDY. 1947. Studies in the biology of the skin and fleece of sheep. 4. The hair follicle group and its topographical variations in the skin of the Merino foetus. Coun. Sci. Ind. Res. (Aust.) Bull. **215:** 5.
7. CARTER, H. B., H. R. MARSTON, & A. W. PEIRCE. Unpublished data.
8. FERGUSON, K. A., H. B. CARTER & M. H. HARDY. 1949. Studies of comparative fleece growth in sheep. Aust. J. Sci. Res. B **2:** 42.
9. FERGUSON, K. A., H. B. CARTER, M. H. HARDY, & H. N. TURNER. Unpublished data.

HAIR PATTERN AND DISTRIBUTION IN MAMMALS

By Jerome P. Parnell
Department of Anatomy, State University Medical Center at New York City,
Brooklyn, New York

In reviewing the subject of hair pattern and distribution, one is concerned with the length of the hair, its direction, and whether it is abundant or scanty. These characteristics of the hair coat are relatively constant for a given species, but there is great variation when one considers the whole class of mammals. This is evident in the diversity of hair pattern that is found in various domesticated animals. The relatively uniform pattern that is found in the common rodent is thought by some workers to represent an early or primitive type. The length of the hair in such an animal is relatively constant. The hair on the dorsum is longer than on the venter, and it is short on the extremities, head, and ears. The hair slopes backward from the head to the tail, and on the limbs from proximal to distal. This simple hair pattern has few reversals, no whorls, *etc*., and the hair is abundant over the whole body of the animal. In examining many mammals, we find great variation from this pattern. The development of hair whorls, featherings, and crests, with which Kidd (1901) was most concerned, show the extremes of variation in hair slope. The development of hair tufts, which may be present on the animal's chin, neck, or between the forelimbs, as well as the development of mane hair, are variations in hair length. Of all the variations in animal hair pattern, hair slope is probably the most investigated.

Osiander (1816) was probably the first to investigate hair direction in human embryos. His investigations led him to the conclusion that the hair inclination, in general, followed the course of the arteries.

Voight (1857) found that the hair direction was established during the development of the hair germ. He said that, since the distal end of the hair was more fixed by dense connective tissue than the proximal end, the hairs were directed in a sloping fashion with growth of the body. He believed that the change from the perpendicular to the sloping condition of the hair germs was produced by the stretching of the skin during development.

Kidd (1901), in his book on hair direction in mammals and man, gave an evolutionary, or Lamarckian, explanation of hair direction, and, although his theories were later disproved, his descriptions and photographs of hair whorls, featherings, crests, *etc*., in various mammals are still useful.

Kidd's hypothesis embraced three basic rules or laws: (1) that there is a basic or primitive pattern of hair direction (the hair, in general, slopes from the tip of the animal's snout backward to his tail, and on the limbs downward from proximal to distal); (2) that morphological changes in the animal can cause changes in this basic plan; and (3) that all other changes in hair direction are brought about by the habits of the animal or the mechanical stress offered by muscles.

Kidd's observations on hair pattern were probably the stimuli for many other workers. The work that followed took two general trends: the first was an explanation of hair slope from an evolutionary viewpoint. This should have expired with the proof that acquired characteristics were not inherited, but, even as late as 1925, Jones (1925) attributed hair reversals in the kangaroo to the scratching and toilet habits of the animal. He said, "Long before the pouch embryo has need to scratch itself, its coat bears the impress of the scratching done in the idle moments of its ancestors."

The other trend looked toward ontogeny for the explanation of hair slope.

The next most significant work is that of Landauer (1925). In his study of hair direction in the opossum, he found that a sloping condition makes its appearance in the early primordia of the hair, and is due to a number of mechanical and physical forces acting at that time. He claimed that, in mammalian development, there is a period, before the development of hair, when the skin is loose. It is during this period that the sense hairs make their appearance and have a straight course. Then, there is a period when the development of deeper structures is accelerated and, because of this, the skin with its developing hair is stretched; consequently, the hair has a tendency to slope.

If this hypothesis is true, said Landauer, then it should be true that the thinner the skin, the smaller the angle between the hair and the skin surface. Here he cites the work of Frédéric (1906). Frédéric measured the scalps of individuals of several races of people and showed a positive correlation between the thickness of the cutis and the angle of the hairs with the cutis. His data are not convincing, however, because he examined only 19 cases. Upham and Landauer (1935) studied samples of the vertex of scalps obtained from 101 individuals on second-day postmortems. They measured the thickness of the cutis and subcutis, and the hair slope. They found that, in females, the average thickness of cutis is smaller, subcutis larger, and the hair slope lesser, than in the male. They also found a significant positive correlation between thickness of cutis and the angle of the hairs with the cutis. Another physical relationship that should be true, if his theory is correct, is that the thicker the hair, the more erect it should stand. Here he cites the work of Lehman (1920) who found that, in the sheep, the downy fibers, which have a diameter of 9–45 micra, lie nearly flat, while the kemp, with the thickness of 50–150 micra, are nearly perpendicular.

Landauer explained the problem of vortices and whorls (roughness of the hair) as centers of forces of strain which tend to disturb the normal pattern.

Castle (1905) and Wright (1916) found that the roughness, or vortices, in the guinea pig were inherited traits that were the result of the interaction of two pairs of genes, a dominant gene (R) and a modifying gene (m).

The work of Wright (1935), Nordby (1932), and others proved very definitely that some complex hair patterns, such as whorls, are inherited. Bolk (1924), in his study of hair slope in the frontal region of human fetuses, describes complex patterns, whose causality cannot be explained by the influence of the external environment, as suggested by Voight, and must be the result of a complex inherited pattern.

On the other hand, other investigators emphasized the importance of mechanical forces acting on the individual during development as the principal element in determining hair slope. To determine the importance of these factors, they employed a new tool, that of experimental manipulation of the skin.

Trotter and Dawson (1932) found that some change in hair direction resulted after a piece of skin was rotated 180 degrees in the newborn rat. A similar experiment was performed on the rat by Butcher (1936), who found that the resulting skin hairs had a mixed direction.

David (1934) sewed a tuck in the skin of one- or two-day-old mice. After five or six days, the hair on the posterior fold pointed toward the head, due to a reversal, caused by a change in the tension on the skin. She also showed changes in grafts that were rotated, and attributed these to a change in the direction of stress.

Colin (1943) studied the embryogenesis of hair follicles in the guinea-pig and feels that factors such as tension, pressure, and thickness of the skin, operative after birth, could not possibly explain the factors causing hair direction in the primordia of the embryo. Based on his microscopic observations of hair primordia in guinea-pig embryos of various ages, he feels that the sloping condition of the hair is a product of differential growth of the developing hair follicle. That is, there is a more rapid growth on one side of the follicle than on the other. He suggests that the more actively growing regions may possibly give off a growth-promoting substance which sets up a physiological gradient. According to this author, centers of low metabolic activity should then represent the center of hair whorls. He claims that the developing hair follicle must act as an organizer for other follicles, since the first follicle penetrates a short distance into the dermis and sets up satellites which appear on either side of the original. Later, a second pair appears, and so on, until sets of 8 predominate, as pointed out by Dawson (1930).

Thus, it seems that the factors that are responsible for hair slope fall into two possible categories. The first stresses those extrinsic factors which influence the growth of the embryo, such as tension and pressure on the skin surface, its thickness, and the presence or absence of the structures beneath it. The second emphasizes the importance of intrinsic factors, either in the growing hair germ, such as a physiological gradient, or in the genetical makeup of the individual.

The relative value of these forces in the production of hair slope and pattern can only be determined by further investigation and experimentation. To establish such things as the relationship between hair slope and hair thickness on the one hand, and slope and skin thickness on the other, would be a step in the right direction. Possible direct evidence for the existence of a physiological skin gradient may help to answer this problem.

Bibliography

Bassett, C. & M. Leonard. 1948. The molting and fur growth pattern in the adult silver fox. Am. Midland Nat. **39:** 597–601.

Bolk, L. 1923–24. On the hair slope in the frontal region of man. J. Anat. **58:** 206–221.

Brandt, W. 1940. The prenatal development of the hair tracts in primates. Human Biol. **12:** 203–231.

Butcher, E. 1936. Hair growth on skin transplants in the immature albino rat. Anat. Rec. **64:** 161–171.

Castle, W. E. 1905. Heredity of coat characters in guinea-pigs and rabbits. Carnegie Institution Washington Pub. 23.

Child, C. M. 1941. Patterns and Problems of Development. The University of Chicago Press, Chicago.

Colin, E. C. 1943. Hair direction in mammals; embryogenesis of hair follicles in the guinea-pig. J. Morphol. **72:** 191–223.

Craft, W. A. 1932. The inheritance of hair whorls (in swine). Proc. Okla. Acad. Sci. **12:** 7–8.

Craft, W. A. & E. A. Warner. 1934. Observations on different types of hair whorls in mammals, and the inheritance of hair whorls in swine. Proc. Okla. Acad. Sci. **14:** 23–27.

David, L. T. 1931. Hairless mammals; comparative histologic studies. Arch. f. Dermat. u. Syph. Berlin.

David, L. T. 1934. Modification of hair direction and slope on mice and rats (*Mus musculus* and *Mus norvegicus albinus*). J. Exper. Zool. **68:** 519–528.

Dawson, H. L. 1930. A study of hair growth in the guinea-pig (*Cavia cobaya*). Am. J. Anat. **45:** 461–484.

Fraser, D. A. 1928. The development of the skin of the back of the albino rat until the eruption of the first hairs. Anat. Rec. **38:** 203–223.

Frédéric, J. 1906. Untersuchungen über die Rassenunterschiede der menschlichen Kopfhaare. Ztschr. f. Morphol. u. Anthropol. **9:** 248–324.

Glaister, J. 1931. Study of hairs and wools . . . mammalian groups . . . human hair. Egyptian Univ. Facul. Med. (Pub. 2): 1–187.

Hale, C. W. 1945. Colors and growth of hair in rabbits. Nature **155:** 670–671.

Hellinga, G. 1946. Hair growth in rats. Acta brev. Neerland. **14:** 83–86.

Jones, F. W. 1924. On the causation of certain hair tracts. J. Anat. **59:** 72–79.

Jones, F. W. 1925. The hair pattern of a kangaroo; a study of cause and effect. J. Mamm. **6:** 13–17.

Kidd, W. 1901. Use-inheritance Illustrated by the Direction of Hair on the Bodies of Animals. Adam and Charles Black, London.

Kidd, W. 1901. Notes on the hair slope in man. J. Anat. & Physiol. **35:** 15.

Kidd, W. 1903. The Direction of Hair in Animals and Man. Adam and Charles Black, London.

Landauer, W. 1925. On the hair direction in mammals. J. Mamm. **6:** 217–233.

Lehmann, C. 1920. Bedeutung und Hauptgrundlinien der Wollkunde, in: Heymons, Lehmann, Volz, und Freyer, Fragen der Schaftzucht, II. Arb. d. Deutschen Landwirtschaft-gesellschaft, Heft 306.

Miller, G. S., Jr. 1931. Human hair and primate patterning. Smithsonian Misc. Coll. **85:** 1–13.

Niedoba, T. 1917–18. Untersuchungen über die Haarrichtung der Haussäugetiere. Anat. Anz. **50.**

Nordby, J. E. 1932. Inheritance of whorls in the hair of swine. J. Hered. **23:** 397–404.

Okamura, T. 1899. Zur Lehre über die Wachstumsrichtung der Haare in der ersten Anlage. Monatschr. f. prakt. Dermat. **28.**

Osiander. 1816–18. De homine quomodo formetur continuatae observationes, spectantes inprimis epidermidem, cutem et pilos fetuum. Commentationes societatis regiae scientiarum Goettingensis recentiores. **4.**

Schultz, A. 1931. The density of hair in primates. Human Biol. **3:** 303–321.

Schwalbe, G. 1895. Entstehung des Haarkleides bei den Säugetieren. Bericht des naturwissenschaftlich-medizinischen Vereins. Strassburg.

Schwalbe, G. 1909. Über die Richtung der Haare bei den Säugetieren speziell beim Menschen. Münchner Medizinische Wochenschrift.

Schwalbe, G. 1911. Über die Richtung der Haare bei den Affenembryonen, nebst allgemeinen Erörterungen über die Ursachen der Haarrichtungen, in: Selenka, Studien zur Entwicklungsgeschichte der Tiere, 10. Lieferung, Heft 15, Wiesbaden.

Stoss. 1906. Die äussere Bedeckung (*Integumentum commune*) mit Einschluss des Epithelgewebes, in: W. Ellenberger, Handbuch der vergleichenden mikroskopischen Anatomie der Haustiere, Berlin. **1.**

Trotter, M. & H. L. Dawson. 1932. The direction of hair after rotation of skin in the new-born albino rat; a second experiment on hair slope. Anat. Rec. **53:** 19–30.

Trotter, M. 1938. A review of the classifications of hair. Am. J. Phys. Anthropol. **24:** 105–126.

UPHAM, E. & W. LANDAUER. 1935. The relation of thickness of the cutis and the subcutis to hair slope in human skin. Anat. Rec. **61:** 359–366.

VOIGT, C. A. 1857. Abhandlung über die Richtung der Haare am menschlichen Körper. Denkschriften der Wiener Akademie der Wissenschaften, Math.-naturwiss. Klass. **13.**

WRIGHT, S. 1934. Genetics of abnormal growth in the guinea-pigs. Cold Spring Harbor Symposia. **2:** 137–147.

WRIGHT, S. 1935. On the genetics of rosette pattern in guinea-pigs. Genetica. **17:** 547–560.

TYPES AND DISTRIBUTION OF THE HAIR IN MAN

By Stanley Marion Garn
Harvard University, Cambridge, Mass.

Unlike other taxonomic criteria, hair can be used both to compare man to other primates, and to compare sub-groups of the genus *Homo* to each other. In the amount of hair, and the number of morphological types of hair, man differs from all other primates, while in the form, distribution, and development of the body hair, racial differences are great. It is not surprising, therefore, that most racial taxonomies have used hair form and distribution,[1] and some classifications (like Deniker's) have made hair the principal criterion of race.[2] As Weidenreich has pointed out, human taxonomies are unique in the importance given to hair,[3] yet no other characteristic that has been employed is more suitable as a sorting criterion.

Although the major racial differences in hair form and distribution have been known for a long time, our present knowledge of the form, structure, and distribution is largely limited to the hair of the face, head, and extremities. Until recently, few comparative studies had included data on the hair of the trunk, and its patterns. Though physical anthropologists have rated hairiness, the categories have remained undefined, and the entire body was rarely included in such ratings.

Recently, however, it has become practicable to examine and rate the nude subject, and the attention of anthropologists has therefore shifted from the face and head to the entire body. The pioneer quantitative study of body hair, conducted by Danforth and Trotter[4] over twenty years ago, has been followed by the recent investigation of Dupertuis, Atkinson, and Elftman,[5] and studies by the author.[6, 7] These investigations have developed standardized methods for rating body hair, both directly and from photographs. During this time, child-growth studies have contributed data on the growth and development of the adult hair pattern. The appearance of pubic hair, and the development of body hair at puberty has been studied by Greulich in boys[8] and by Pryor in girls[9] and has been summarized by Stuart.[10] Reynolds and Wines have considered the relationship between pubic hair development and maturation,[11] while the relationships between body hair, physique, and sexual dimorphism in the adolescent and young adult have been treated by Bullen and Hardy,[12] Sheldon, Tucker, and Stevens,[13] Bayley and Bayer,[14] and others.

Since the studies mentioned have been conducted on draftees, adolescents, and college students in this country, published data is largely confined to young American whites. Now, due to the current interest in body-build, there are available extensive collections of somatotype photographs taken during medical and anthropological examinations, and these photographs are suitable for comparative racial studies of body hair, as well as investigations of hair changes in older age groups. Body-build photographs now on file include extensive series of American whites, Negroes, Chinese, Japanese,[15] Navajo,[16] Aleut,[17] and Micronesians.[18] Reports on the body-hair distribution of two of these groups will be available in the near future.[19, 20]

Scope of This Paper. As indicated in the title, this paper is concerned with the types of hair in man, as variously defined, and with differences in distribution, density, and patterning. In discussing types, various classifications are considered, and a morphological classification of the adult terminal hair is given. The distribution and patterning of the body hair is approached through the definition of body hair regions, by describing some of the hair patterns within these regions, and by data on hair density and distribution. Knowledge of the different types of hair in man is of use to both the comparative anatomist and the medico-legal expert, while data on the distribution of body hair, and the patterns of body hair in man, is of use to the plastic surgeon and to the endocrinologist, as well as to the student of human races.

Classification of Hair Types in Man. Hair in man can be classified according to form, as reviewed in detail by Trotter,[21] or according to morphological and structural type, as described by Danforth.[22] The criteria employed in dividing hair into morphological types are (1) gross size, (2) time of appearance, and (3) structural variations.

The basic division of human hair into two polar types, vellus hair and terminal hair, depends largely on gross size. The vellus hair is the short, fine, often unpigmented and unmedullated hair that is called fuzz by the layman and often inappropriately termed lanugo hair in the medical literature. The terminal hair, on the other hand, is defined as the longer, coarser, usually pigmented and medullated hair with larger roots and shorter, blunter tips. Though the vellus hair has been compared to the under-fur of other mammals, and the terminal hair to the guard-hairs of these animals, this specialization of types does not occur in man. In several regions of the body, the first hair produced may be vellus in type, while later hair shafts produced by the same follicles are unquestionably terminal hair in type. Because some hairs exhibit characteristics of both types, a third, or intermediate, type has been recognized.

Those working on the foetus, or the infant, find it useful to distinguish between primary, secondary, and tertiary hair.[22] The distinctions are partly temporal in nature, and partly based on differences in length, cross-section form, and thickness. As Duggins and Trotter have shown, there are marked changes in the cross-section characteristics of the head hair during the first post-natal year, and these changes may be explained as the gradual replacement of the lanugo or primary hair by the secondary, or vellus hair, and the terminal hair.[23] Again, it does not appear that different follicles are involved, but rather that changes in the internal environment are reflected by alterations in follicular activity and output.

In the adult individual, terminal hair exists on many parts of the body, and this hair shows regional differences in morphology that are due to inherent, genetically-determined differences in the follicles themselves. Thus, skin grafts in plastic surgery continue to produce shafts characteristic of the donor site. In addition to the morphological differences characteristic of hair from different parts of the body, the follicles themselves are highly specific to trophic hormones. As a supplement to the morphological classification of hair types, it is possible to devise a hormonal classification, based on qualitative and quantitative differences in the hormones needed either to "trigger" or to maintain growth.[24]

In the hormonal classification, it is possible at present to distinguish three types of hair:

(1) Hair not dependent upon steroid hormones, but influenced by changes in growth-hormone output and sometimes inhibited by androgenic hormones: the hair of the head, the eyelashes and eyebrows, the mid-phalangeal hair, hair on the distal segments of the limbs, and hair on the lumbosacral triangle to some extent.

(2) Hair dependent upon female amounts of steroid hormones: pubic and axillary hair, the hair upon the limbs, and (to a lesser extent) hypogastric hair.

(3) Hair dependent upon male amounts of steroid hormones; beard and moustache hair, nasal-tip hair, ear hair, and body hair (especially on the back).

The morphological differences are largely evident to the naked eye, and by combining macroscopic and microscopic differences, employing the criteria of length, thickness, and size of root and tip, as well as structural variations, it is possible to distinguish six morphological types of hair, referable to comparable follicular types. Of these six morphological types, the distinctions are least pronounced between types I and II, and V and VI. The six morphological types and their principal criteria are given in TABLE 1.

It will be noted that the hormonal classification results in three types, while the morphological classification provides six types. The difference in number may be due to the fact that one hormone may stimulate several classes of follicles. It is possible, however, that further fractionation of the steroid hormones will reveal steroids specific to the pubic, axillary, and body hair respectively.

The morphological classification presented here exemplifies the difference between man and lower mammals. In lower mammals, the terminal hair is more uniform in type, except for the tactile bristles. In the primates, there is a tendency for diversification of type as well as marked hormone-mediated sex differences. In the anthropoids, both diversification of types and the specificity of follicles is most marked.

The Distribution of Body Hair in Man. These morphological types of hair are present in nearly all adult human males. Of these six types, the head hair, the eyebrow and eyelash hair, the pubic hair, and the axillary hair do not exhibit enough known variations in amount and patterning to make them of immediate use in racial studies. The beard and moustache hair, on the other hand, and the body hair to a greater extent, show marked variations in density, distribution, and patterning, and these latter types can be used in endocrine studies, in investigation of growth and aging, and in racial studies. Moreover, it is important for the plastic surgeon to know what regions of the body are apt to bear terminal hair in the adult, if the embarrassment and discomfort resulting from the injudicious selection of grafts is to be avoided.

In conducting studies of body hair distribution, three variables are to be considered: (1) location, (2) pattern, and (3) density. Location refers to where the hair grows, pattern refers to the configuration within those areas, and density refers to the amount of hair on each location.

TABLE 1
MORPHOLOGICAL TYPES OF HAIR IN MAN[7]

Type	*Metric characteristics (normal range)*	*Description*
I. Head hair	100 to 1000 mm. in length, cross section diameter @ 25 to 125 μ	Relatively small root, tapered tip, usually medullated, all variations in form known.
II. Eyebrow and eyelash hair	Average length about 1 cm.	Curved, coarse, smooth with punctate tip and large medulla.
III. Beard and moustache hair	Length 50 to 300 mm.	Larger root than I, more complex medullary processes, more irregular structure, blunter tip.
IV. Body hair	3 to 60 mm. in length	Fine long tip, irregularly medullated, irregularities in structure; may be banded or tipped. All forms and structural types.
V. Pubic hair	10 to 60 mm. in length	Coarse, irregular and asymmetrical cross section with many constrictions and twists, usually curved but may be straight or spiral-tufted.
VI. Axillary hair	10 to 50 mm. in length	Coarse. Usually straighter than V, but may be spiral-tuft in the Negro. Tip blunt, usually abraded and split, many cortical fusi.

In earlier works, the author was interested in devising a rating system for body hair and attempted to determine the minimum number of hair regions that should be studied separately, defining a region as an area of the body where hair growth is at least partially independent of terminal hair on neighboring regions.[6, 7] After inspection of 2,600 body-build photographs, nine such regions were listed. These regions were: (1) beard and moustache, (2) hypogastric, (3) thoracic, (4) lower arm and leg, (5) upper arm and leg, (6) gluteal, (7) lumbo-sacral, (8) lower back, and (9) upper back. These regions exhibited (a) regional independence, (b) considerable racial and individual variation in pattern, and (c) individual and racial variations in density.

Subsequently it has been necessary to add two more regions: (10) the mid-phalangeal region, and (11) ear hair. The mid-phalangeal or "mid-digital hair" of Danforth[25] has a simple mode of inheritance,[26] and the gene frequencies vary in different racial groups.[27] Ear hair, as described by Wallis[28] and Danforth[22] is usually present in the newborn,[7] is absent in the child and the young adult, and is limited, in later life, to the male.[29] (See FIGURE 1 and TABLE 2.)

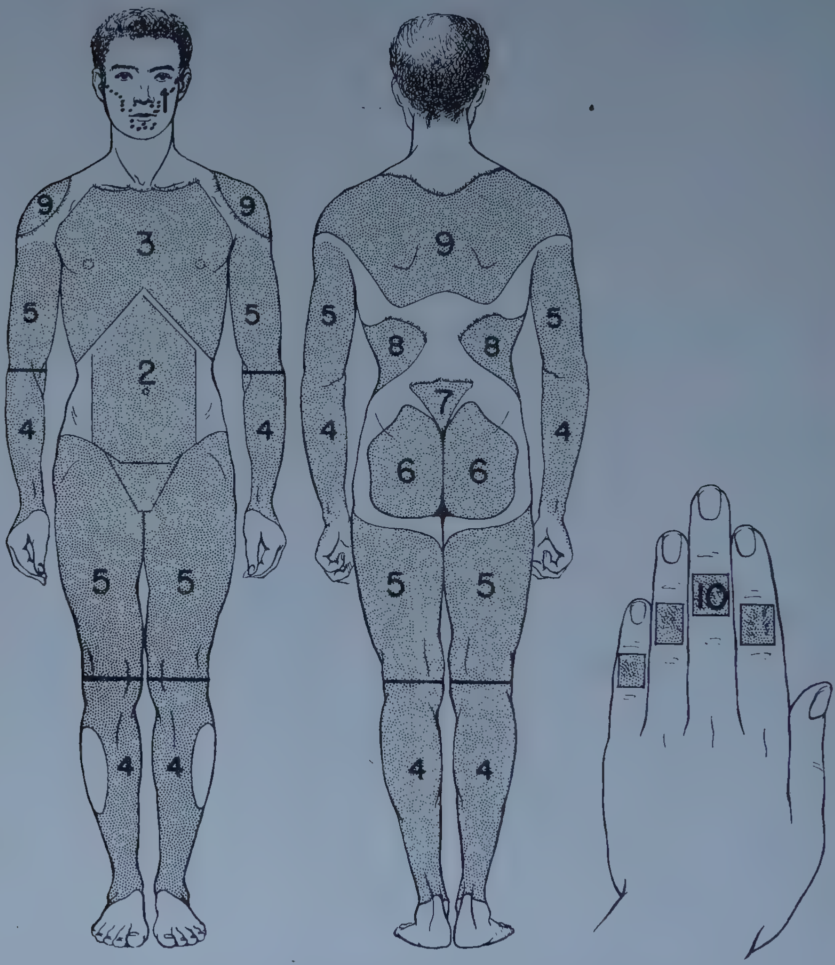


FIGURE 1. The body-hair regions in man, as defined in the text: (1), beard; (2), hypogastric; (3), thoracic; (4), lower arm and leg; (5), upper arm and leg; (6), gluteal; (7), lumbo-sacral; (8), lower back; (9), upper back; (10), mid-phalangeal. Region 11 (ear hair) not shown. The outlines delimit the approximate maximum extent of the various regions, especially regions 8 and 9. Region 10 is shown as a square shaded area, since the outline of this region has not yet been determined. In the majority of males, only part of a region bears terminal hair (see text).

It should be emphasized that many of the patterns given in TABLE 2 are transient, and change with age, especially during the later teens and twenties. Danforth,[22] and Dupertuis and his co-workers[5] noted that the frequency of specific patterns varied with age. Thus, a youth with the linea pattern of hypogastric hair at 16 may have a pyramidal pattern at 20, and a complete rectus coverage at 40.

Hair Distribution by Regions. TABLE 3 is included to provide an indication of the frequency of appearance of hair on each region. The data is based on the author's observations on a series of 239 white American males, with a mean age of 39, examined during the course of medical and anthropological studies.[30] Ratings were made directly on the nude subjects, at a standard viewing distance of 10 feet (except for region 10), and under 150 foot-lamberts of illumination. Density was rated on a scale of zero to four as suggested by Danforth and Trotter.[4] Zero referred to absence of terminal hair, or a few terminal hairs, while a rating of 4 indicated a thick, dense mat. For region 10, the original method of Danforth[25] was followed and the fingers bearing mid-phalangeal hair were recorded separately.

TABLE 2
BODY HAIR REGIONS IN MAN

Region	*Range of patterns*
1. Beard and moustache	"Amerindian" pattern of sparse moustache and isolated chin-beard hairs to "Mosaic" pattern with fully confluent facial hair.
2. Hypogastric	"Linea" pattern to the navel, pyramidal pattern,[5] and finally to complete coverage of m. rectus abdominis confluent with region 3.
3. Thoracic	Circum-areolar and sternal tuft, to mat confluent with deltoid hair, axillary hair, and regions 2 and 9.
4. Lower leg and arm	Distal on extremities, to entire coverage. Balding pattern on calves.
5. Upper leg and arm	Streams confluent with 4 and 9.
6. Gluteal	Perianal band, to full coverage of gluteal region and confluence with regions 5 and 7.
7. Lumbo-sacral	Patch triangular in form, varying in density. May be present prior to puberty.
8. Lower back	Infrascapular arcs, to confluence with regions 6, 7, and 9.
9. Upper back	Shoulder band, to complete "T shirt" pattern confluent with regions 3 and 8.
10. Mid-phalangeal	Varies in number of fingers (most usually digit IV) and in density per square centimeter.
11. Ear hair	Tuft at tragus, at Darwin's point, band on helix and arc at concha. Marked racial differences in expressivity.

TABLE 3* gives the frequency of appearance of terminal hair on each region, and the modal density on that region. As would be expected, the lower arms and legs, which exhibit terminal hair even in the child, most frequently exhibit terminal hair in the older adult male, though the density is greater in the adult. On the trunk, the ventral aspect is considerably hairier than the dorsal aspect: hypogastric hair was present in 79 per cent. Unpublished data collected by the author indicates that a comparable proportion of young adults exhibit hypogastric hair, but a much smaller proportion exhibit thoracic hair.[30] In contrast to the hairiness of the abdomen and thorax, no dorsal hair region exhibited terminal hair in more than half of the individuals: 43 per cent showed gluteal hair, 28 per cent had hair on the lower back, and 25 per cent had hair on the upper back.

TABLE 3

FREQUENCY OF APPEARANCE OF TERMINAL HAIR (239 ADULT WHITE MALES)

Region	*No.*	*Frequency of appearance**	*Modal† value*
1. Beard	239	1.00	2
2. Abdominal	239	.84	1
3. Thoracic	239	.79	1
4. Lower arm, leg	239	.97	2
5. Upper arm, leg	239	.85	1
6. Gluteal	239	.37	0
7. Sacral	239	.43	0
8. Lower back	239	.28	0
9. Upper back	239	.25	0
10. Mid-phalangeal	239	.67	0, 1

* One degree or more.
† rated on a scale of zero to four.

Since this series was rated by direct observation of the subjects, rather than from photographs, the data is not comparable with that of Dupertuis *et al.*,[5] but is comparable to and consistent with the findings of Danforth and Trotter.[4]

The evidence that man is hairier on the thorax than on the back is also in concordance with the observations of Schultz,[31] who observed that the thoracic region of the gorilla showed a tendency toward hair reduction, while the chests of the human males in his series were relatively and even absolutely hairier. While Darwin suggested that man's relative hairiness came about from the preferential selection of females with less ventral hair (and therefore greater sexual stimulus value) in paleanthropic times,[32] it would appear that the reduction of hair in man is actually more pronounced on the back.

The data presented here also suggest that the selection of sites for donor grafts in plastic surgery must be made with care, especially in younger individuals. Since the majority of adult males exhibit considerable terminal hair on the abdomen and thorax, these regions should be avoided if the skin flaps are to be grafted onto the face, into the oral cavity, or into the nasal cavity, for troublesome later hair growth may be anticipated.

---

* The data included in TABLES 3, 4, and 5 are derived in part from studies financed by a grant from the Commonwealth Fund of New York, and were gathered during the examination of patients and control subjects. The author expresses his appreciation to Dr. Paul D. White and the advisory board of the Coronary Research Project for permission to publish. Dr. M. M. Gertler helped to improve the method and to standardize the ratings.

Rating Hirsutism in Man. Since the system of rating body hair just described includes both the presence or absence and the amount of hair present on each of 11 regions of the body, it is possible to use the data in any one of three ways, when comparing groups of individuals or in determining whether a given individual is relatively glabrous or relatively hairy. First, in contrasting groups of individuals, it is possible to compare the frequency of appearance of terminal hair on each of the regions of the body, as well as the mean or modal amount of hair on each region, as shown in TABLE 3. Second, it is possible to sum up the total number of regions bearing terminal hair, and to express hirsutism either as this sum, or the percentage of all regions bearing hair. Thus, with 11 regions, the theoretical range would be 0 to 11, and the theoretical range of percentages would be 0 to 100 per cent. Last of all, it is possible to sum up the total amount of hair, by regions, for all regions: with a range of 0 to 4 for each region, and 10 regions in all, the theoretical range would be 0 to 40.

TABLE 4

HIRSUTISM BY NUMBER OF REGIONS—239 MALES

Number of regions bearing terminal hair	*Number of individuals*	*Frequency per 100*
1	1	0.4
2	10	4.2
3	16	6.7
4	17	7.1
5	33	13.8
6	60	25.1
7	23	9.6
8	25	10.5
9	30	12.6
10*	24	10.0
	Total no. 239	Total 100.0

* Region 11 not rated in this study.

The first method, that of stating the frequency of appearance of body hair on each region, is most suited to racial comparisons. It provides a differential description, but does not express the overall hairiness of a group. The second method, that of expressing the total number of areas bearing terminal hair, with a theoretical range of 0 to 11, may be satisfactory for females, and for relatively glabrous races, but is not satisfactory for the males described in this study. Instead of a normal curve, as shown in TABLE 4, there was a strikingly skewed distribution, and the two maximum scores were attained by 22.6 per cent of the group. Hence, the system of expressing the total number of regions bearing terminal hair does not distinguish sufficiently well between different grades of hirsutism, neglecting as it does the total amount of hair.

As shown in TABLE 5, the third method, that of summing up the total amount of hair by regions, for all regions, gives the most satisfactory result. With a theoretical range of 0 to 40 (region 11 was not rated in this group), the actual range observed was 3 to 33. Individuals with a score of 5 or less are the glabrous ones, for this particular series, while males with a score of 18 or more are the hairy males, for this particular series. Individuals scoring 8 or 9 have a modal amount of hair. In contrast to this distribution, the mean score for a small series of Aleut males was 3, a value below that of a pilot series of American women. Hence the technique of rating hirsutism in terms of the total amount of hair, for all regions, seems to be useful. Obviously the ratings are to some degree subjective, but less subjective than many methods still in use, and the observer does not have to rely on his own idea of what an average degree of hairiness is.

TABLE 5
FREQUENCY DISTRIBUTION, TOTAL AMOUNT OF HAIR (239 ADULT WHITE MALES)

Amount	*No. of individuals*	*Freq. per 100*
0, 1	0	—
2, 3	10	4.4
4, 5	18	7.5
6, 7	32	13.4
8, 9	44	18.4
10, 11	34	14.2
12, 13	23	9.6
14, 15	20	8.4
16, 17	13	5.4
18, 19	12	5.0
20, 21	12	5.0
22, 23	7	2.9
24, 25	1	.4
26, 27	5	2.1
28, 29	1	.4
30, 31	5	2.1
32, 33	2	.8
	Total no. 239	Total freq. 100.0

Future Prospects for Research. As stated previously, little has been done on the patterns of body hair, on hirsutism, on the relative hirsutism of various races, and on the changes in body hair associated with age. If observers note, during their studies, (1) the regions that bear terminal hair, (2) the amount of terminal hair on each region, and (3) the patterning of the hair on each region, then a body of data will be accumulated for racial and comparative studies. It is very likely that the mode of inheritance of some of the patterns of body hair can be determined, just as Bernstein and Burks[26, 27] and others have analyzed the inheritance of "mid-digital hair." When this is done, hair will play an important role in the present genetic approach to human taxonomy.

Bibliography

1. Coon, C. S., S. M. Garn, & J. Birdsell. 1950. Races. C. C. Thomas. Springfield.
2. Kroeber, A. L. 1948. Anthropology. Harcourt, Brace. New York.
3. Weidenreich, F. 1946. Am. J. Phys. Anthrop. N.S. **4:** 413–432.
4. Danforth, C. H. & M. Trotter. 1922. Am. J. Phys. Anthrop. **5:** 259–265.
5. Dupertuis, C. W., W. B. Atkinson, & H. Elftman. 1945. Human Biol. **17:** 137–142.
6. Garn, S. M. 1947. Am. J. Phys. Anthrop. N.S. **5:** 241. (Abstract).
7. Garn, S. M. 1948. Human hair: Its composition, anatomy and distribution. Doctorate thesis. Harvard University, Cambridge, Mass.
8. Greulich, W. W. 1942. Monograph. Soc. for Res. in Child. Dev. **7:** 3.
9. Pryor, H. B. 1936. J. Pediat. **8:** 52–62.
10. Stuart, H. C. 1946. N. E. J. Med. **234:** 666–672, 693–700, 732–738.
11. Reynolds, E. L. & J. V. Wines. 1948. Am. J. Dis. Child. **75:** 329–350.
12. Bullen, A. K. & H. L. Hardy. 1946. Am. J. Phys. Anthrop. N.S. **4:** 37–65.
13. Sheldon, W. H., S. S. Stevens, & W. H. Tucker. 1940. Varieties of Human Physique. Harper & Bros. New York. pp. 78–79.
14. Bayley, N. & L. M. Bayer. 1946. Am. J. Phys. Anthrop. N.S. **4:** 433–460.
15. Photographed by B. L. Kraus.
16. Photographed by James N. Spuhler.
17. Photographed by Charles I. Shade and Stanley M. Garn.
18. Photographed by E. E. Hunt.
19. Hunt, E. E. 1950. A somatological study of the Micronesians of Yap. In preparation.
20. Garn, S. M. 1950. J. Hered. **41.** In press.
21. Trotter, M. 1938. Am. J. Phys. Anthrop. **24:** 105–128.
22. Danforth, C. H. 1925. Hair. Am. Med. Assn. Press. Chicago.
23. Trotter, M. & O. H. Duggins. 1948. Am. J. Phys. Anthrop. N.S. **6:** 489–506.
24. Garn, S. M. 1948. Am. J. Phys. Anthrop. N.S. **6:** 235. (Abstract).
25. Danforth, C. H. 1921. Am. J. Phys. Anthrop. **4:** 189–204.
26. Bernstein, M. M. & B. S. Burks. 1942. J. Hered. **32:** 45–53.
27. Bernstein, M. M. 1949. J. Hered. **40:** 127–131.
28. Wallis, H. M. 1897. Proc. Zool. Soc. London. II: 298–310.
29. Hamilton, J. B. 1946. Personal communication.
30. Garn, S. M. & M. M. Gertler. 1949. Unpublished data.
31. Schultz, A. H. 1931. Human Biol. **3:** 303–322.
32. Darwin, C. 1898. The Descent of Man, and Selection In Relation to Sex. 2nd ed. Appleton & Company. New York.

DEVELOPMENT OF THE PILARY SYSTEM AND THE REPLACEMENT OF HAIR IN MAMMALS

By Earl O. Butcher

Departments of Anatomy, College of Dentistry and the Graduate School of Arts and Science, New York University, New York, N. Y.

The phenomenon of development attracts the attention of many biologists, who often seek rare materials for solving their problems when the skin offers many opportunities and is so easily observed. Much is known regarding the development of the pilary system. However, several problems remain to be solved. Some information undoubtedly will be presented on these problems in some of the subsequent papers, yet many questions will remain as an incentive for further work.

The Rat and Mouse. The development of the pilary system in our most common laboratory animals, the rat and mouse, will be described first, since many extensive studies have been made on them.[1–4] Hair germs become apparent in 17- to 20-day fetal rats as a crowding of cells, with a slight convexity, in the deepest layers of the epidermis. These germs continue to form from the stratum germinativum from birth until the age of 10 days.

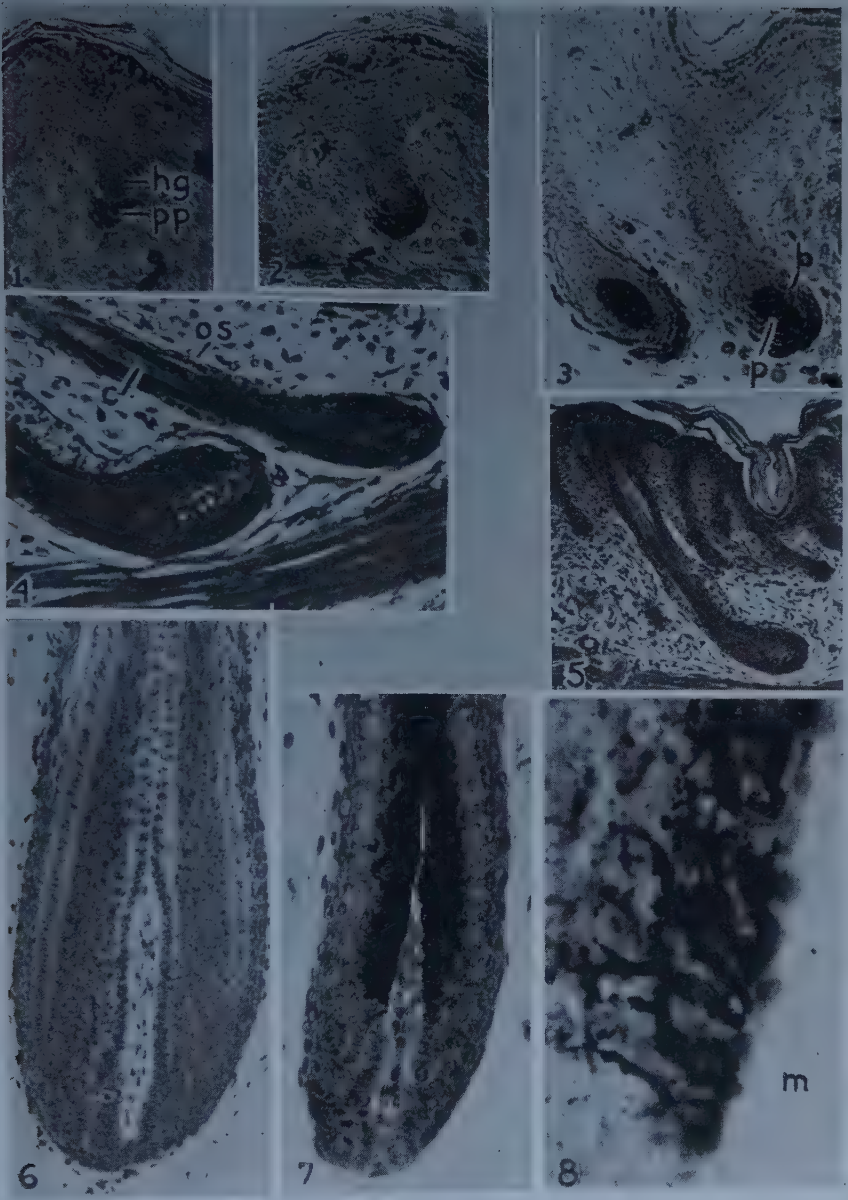
The next stage in their development consists of an increase in their convexity and the appearance of a slight condensation of connective tissue, the prospective papilla, deep to them (FIGURE 1). Further development consista of an elongation of the epithelial bud, whose base is capped by the enlarged prospective papilla (FIGURE 2). In a slightly later stage of development, which occurs typically in the 2-day-old rat, the base of the epithelial portion has enlarged and invaginated to form the bulb of the hair, and the enlarged connective tissue papilla protrudes into the bulb (FIGURE 3).

Beginning near the bulb, the core of the epithelial column separates from the peripheral cells, which become the outer sheath of the hair and retain their continuity with the stratum germinativum (FIGURE 4). The core forms the inner sheath and the shaft of the hair. In 5-day-old rats, some of the epithelial down-growths have few indications of much differentiation, while in many of them may be seen a definite hair shaft with inner and outer root sheaths.

As the hair and its sheath elongate, the central epithelial cells of the column distal to the cone move aside, allowing the tip of the hair to erupt (FIGURE 5). The inner root sheath, by its glassy appearance, contrasts sharply with the outer, especially near the apex where nuclei are absent or scarce. Huxley and Henle layers can soon be recognized in the inner sheath. The epithelial cells of the bulb, resting on the surface of the papilla, represent the future matrix. This matrix, by active mitosis in that portion at the base of the papilla, gives rise to cells destined to become cuticle, cortex, and inner sheath, while the matrix cells around the tip of the papilla are pushed outward to become the medulla of the hair shaft (FIGURE 6).*

---

* The author is indebted to Dr. A. C. Taylor of the Department of Anatomy, New York University, for material for FIGURES 6, 7, 8, 12, 13, 15, 16.



FIGURES 1–8.

FIGURE 1. Section from day-old rat showing hair germ and prospective papilla which contains phosphatase.

FIGURE 2. Elongated bud and enlarged papilla with phosphatase.

FIGURE 3. Section showing newly formed bulb and papilla containing phosphatase.

FIGURE 4. A follicle in which the core is separating from the outer sheath cells.

FIGURE 5. The eruption of the hair.

FIGURE 6. Section showing origin of the cells of the medulla from the matrix cells around the tip of the papilla.

FIGURE 7. The pigment distribution in the hair of a black rat.

FIGURE 8. Melanophores with dendroid processes extending into the cortical cells.

Abbreviations: b—bulb; c—core; fh—first hair; fn—folds in nuclei; hg - hair germ; m—medulla; n—new cells; o—dead cells; os—outer sheath; p—papilla; pp—prospective papilla; and sh—second hair

(See also page 511.)

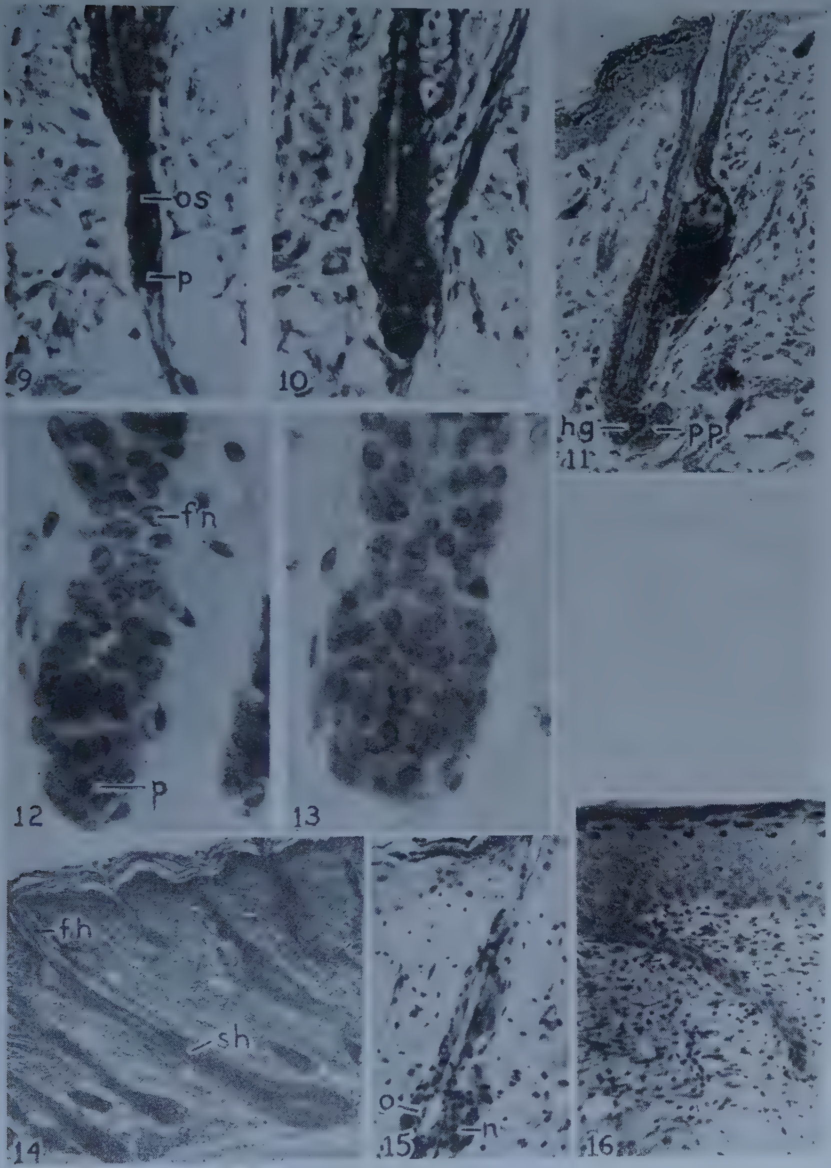
The melanophores of black hair follicles are situated in the matrix, giving rise to the medulla[3] (FIGURE 7). The melanophores are usually larger and contain more spherical nuclei than the other cells of the matrix. Dendroid processes of the melanophores, with their pigment, extend centrifugally into the forming cortical cells (FIGURE 8). Pigment is passed directly into the cytoplasm of the cortical and medullary cells by the pigment cell processes. Cells identical to the melanophores in black hairs are found in white hairs of albino rats and appear to be colorless pigment cells. The white hairs of hooded rats appear to be lacking entirely in pigment cells.

Two types of follicles may be found in the rat's coat. Some are very large and contain the longer and coarser hairs, while the smaller follicles are more numerous and have shorter, finer hairs.

The matrix cells continue to add material to the elongating hair in the rat until the definitive length is reached at about the age of 17 days. In black hairs, the formation of pigment stops a few days before the definitive length is reached, and the diameter of the hair is gradually reduced.

This formation and elongation of the hair, which ends about the 17th day of life, constitutes the growing stage of the hair. At the age of 17 or 18 days, the bulb of the hair begins a rapid transformation into a cornified, club-shaped structure, closely surrounded by a sheath. The hair-club can be seen in the process of formation while the root is still quite long and a larger part of the inner root sheath still remains. The hair-club slowly moves outward, stopping just below the level of the sebaceous gland. Desquamation of the inner root sheath occurs as it moves outward. The empty outer sheath forms an epithelial strand, shortens, and, with the remnants of the papilla, moves outward (FIGURES 9 and 10). The outer root sheath appears to pass into, or is folded against the base of, the hair-club. At this time, between the club sheath and papilla in the mouse, according to Dry, the hair germ of the next hair often becomes recognizable. This transformation into a quiescent condition occurs within an interval of 3 or 4 days and, usually by the 21st day of life, the root of the hair is securely lodged in a resting follicle, which remains inactive until the 31st or 32nd days of life. This inactivity, extending from the 17th to 32nd days, has been designated as the resting period and, with the growth stage, constitutes a hair cycle.

The beginning of the growth stage of the next, or second, cycle, on the 32nd day of life, is marked by the appearance of a more definite hair germ at the base of the resting hair (FIGURE 11). Taylor, in freezing experiments in the rat, finds good evidence that most of the cells of the germ are proliferated from the base of the resting hair. He noted that the cells of the resting bulb had oval nuclei whose surfaces were thrown into folds. When these cells left the old bulb, in the process of forming a new hair germ, the folds in the nuclei persisted. Such folds enabled him to follow the origin of the cells in the new germ (FIGURE 12). Dry, as mentioned above, was able in many instances to recognize the hair germ much earlier in the mouse. The recognition of a hair germ depends upon the quantity of cells at the base of the resting hair. Apparently, in the mouse, as the resting stage is established, a considerable quantity of cells persists, while



FIGURES 9–16.

FIGURE 9. The outer sheath and remnants of the papilla are moving outward.

FIGURE 10. The outer sheath and old papilla have approached the hair-club.

FIGURE 11. The hair germ and prospective papilla of the second hair in a 30-day-old rat.

FIGURE 12. Folds or indentations in the nuclei of cells moving from the base of the old hair into the hair germ.

FIGURE 13. A melanophore with its long processes.

FIGURE 14. A section showing the second hair pushing up beside the first hair.

FIGURE 15. The cells of the old resting bulb were killed in freezing and appear as dense spots, "o." New cells have migrated down from the epidermis and are the cells marked "n."

FIGURE 16. A new bud has formed from the epidermis, independently of a pre-existing hair follicle, in skin that was frozen.

(See page 509 for figure abbreviations.)

in the rat, only a few with growth potentialities remain closely folded at the base of the hair-club. Simultaneously with the development of the hair germ, the prospective papilla appears, which is a condensation of dermal cells.

Among the cells that are contributed from the resting hair to the hair germ, a relatively small number may be distinguished from the rest by the development of long processes and a transparent cytoplasm containing minute melanin granules (FIGURE 13). These are the melanophores and, according to Taylor,[3] they can be traced through consecutive stages to the fully differentiated pigment cells of the growing hair bulb. New pigment cells originate by the division of cells of the original melanophore stock received from the resting hair.

Some workers have taken the view that the same papilla can function in the growth of another hair. This may be true for vibrissae, for another hair is formed as soon as the growth of its predecessor is completed, and the original papilla appears to persist. Dry has been able to follow the papilla in the mouse by the accumulation of residual pigment in it. Just prior to the growth of the hair germ, the old papilla ruptures and a new one forms at the base of the follicle.

Growth is accomplished at first by the addition of more cells, from their respective sources, to those elements of the bud and the bud then enlarges through mitosis, pushing deeper into the dermis. The advancing germ invaginates and envelopes the prospective papilla. Again, the matrix cells, or cells adjacent to the papilla, form a hair cone which separates from an external layer of cells, the external hair sheath.

The new hair usually does not push the old hair from the follicle but grows next to it (FIGURE 14). The fine hairs, or puppy hairs, produced in the first cycle, are thus joined by coarser hairs, which have the same quality, in subsequent cycles. This growing stage, as in the first cycle, lasts about 17 days and is followed by a resting period. Hair cycles in the rat, therefore, occur approximately every 34 days.

The cyclic growth occurs as a wave. Activity in follicles of the venter may be observed two to three days before it occurs in the dorsum. The wave begins in the venter, spreads dorsally, then anteriorly, and posteriorly. In the rat,[11] it has been shown by skin transplantations that the follicles are receptive most of the time. What, then, is the controlling factor in the time of growth and the cause of the wave of growth?

Dry[4] has observed a similar cyclic growth in the mouse. The first growth is completed at the end of a month, the second at two months, and the third sometimes at three months. The fourth generation is sometimes in progress on the venter before the third has finished on the dorsal region. According to him, one is unable always to see the hair germ of the next generation when inactivity, or resting, is being established. The papilla containing residual pigment disappears just prior to growth of the hair germ, and a new papilla is formed in which, with advancing age, residual pigment again accumulates.

Since most of the epithelial hair buds form from the stratum germinativum in the foetus, or soon after birth, the question arises if hair germs may form later in life from the germinativum or, in case of the death of the follicle, from any other source. In the rat, most of the buds are formed prior to the 10th day of life,[2] yet areas denuded by injury often become repopulated. Taylor[3] studied the margins of an area of skin of the rat following lethal freezing. The cells of the hair sheaths and those of the hair bulb had been killed by the freezing, and new cells passed down from the old epidermis through the outer root sheath into the resting bulb for the new hair germ (FIGURE 15). The source of the melanophores was limited, however, to the old hair bulb and, once they were killed, further pigmentation was lacking.

Taylor[3] also observed the formation of new follicles from the stratum germinativum, independently of an old follicle (FIGURE 16). It occurred, however, only when the tissue underlying the new epithelium was old dermis or after newly formed dermis had become completely differentiated. These observations suggest that the differentiation of new hair germs may depend upon some inductive action of the underlying dermis. Hairless areas, resulting from lethal freezing or from transplantation,[5] may thus be repopulated in the rat, and the germinativum may possess hair potentialities throughout most of the life of the rat. Why should the epithelium lose deep growth potentialities while outward growth potentialities persist?

If the possibility of formation of new hair germs from the germinativum exists, one may ask if there is any means of stimulating them. This problem is left for subsequent investigators, who may present evidence that such stimulation may be by irritants, hormones, *etc.* Butcher[6, 7] has presented evidence that the activity of hair germs at the base of resting follicles may be accelerated by adrenalectomy, by thyroxin administration, or by the application of irritants. Confirmation of the effects of adrenalectomy has been made by Ralli and Graef,[8] and Whitaker and Baker[9] have inhibited activity of the hair germ by the percutaneous application of certain adrenal cortical preparations. In the case of irritation, however, one must be extremely careful, because the irritation causing acceleration may cause an acanthosis and parakeratosis in the epidermis.[10]

Alkaline phosphatase has been studied in the developing hair.[12] The enzyme is present to a very slight degree in the early epithelial bud, becoming very abundant in the mesodermal papilla as the follicle elaborates the hair (FIGURES 1, 2, 3). The quiescent period of the hair cycle is characterized by a negligible amount of phosphatase (FIGURE 11). Glycogen, in contrast to phosphatase, is mainly distributed in the epidermal portion of the developing hair.[13]

Mammals Other than Human. The hair cycles have not been followed so closely in other mammals. The weasel and mink,[14] the stoat,[15] the hare,[16] and the ferret[17] have two cycles yearly, since they have spring and autumn molts which consist of the old hairs being pushed out by new hairs. These cycles and the color of the hair can be greatly altered by controlled lighting.[14] It seems appropriate to assume that most of our small wild animals have similar cycles. How much of the cycle is normally consumed by the growth stage has not been determined.

The fox molts only once a year,[18] and this is probably the condition in the horse, although there is popular belief that there are two moltings. Segall[19] has beautifully illustrated the growth and changes in the follicles of the guinea-pig, and Hofer[20] describes hair growth in the cat. Both of these authors include references in their bibliographies to the earlier works on hair growth. Dawson[21] finds that, in the guinea-pig, the average length of the growth stage is four weeks. The majority of the hairs remained in the resting state for twelve weeks in males and non-pregnant females, and fifteen weeks in females which were pregnant. There seems little reliable data for domesticated rabbits.

An interesting problem is how plucking the hair stimulates activity in the hair germ. This is easily illustrated in a gray rabbit when the hair is in the resting period of the cycle. The hair will not be restored on areas which are shaved but, where an area is plucked, pigmentation, which indicates activity in the bulb, can be seen through the layers of the epidermis by gross observations in a few days. The injury, either chemically or by some other means, initiates activity in the hair germ. What is pulled out when a hair is plucked in the growing stage and resting period?

The Human. Growth and replacement of the hairs in the human is similar to the process in the rat. About the only point at issue is whether the hair papilla persists, or a new one forms. This point was discussed at great length by the older investigators. Some were inclined to believe that a new papilla formed while others believed that the new hair develops with the old papilla. Some believed that either might occur. Since the new papilla occupies exactly the same position as the old one, it is difficult to understand why so many writers regard the formation of a totally new structure as essential to the process.

The first epidermal downgrowths of hair rudiments[22, 23] are found between the second and third months of intrauterine life on the upper lip, chin, and supraorbital regions. Between the fourth and fifth months, the hair begins to form in the hair rudiments of the face, and a month later the process of cornification has begun. By the sixth month, a lateral growth of the hair rudiment has evolved into a sebaceous gland, and another lateral outgrowth has formed to give insertion to the fibers of the erector pili muscle.

In the foetus, all the hairs belong to the lanugo type of hair. The shedding of the lanugo hairs *in utero* begins in the face and head of the foetus between the seventh and eighth months, and these hairs are replaced by new hairs which grow out of the old follicles. These new hairs, in certain regions, such as the scalp, are long and stiff, while in the rest of the body new lanugo hairs take their place, forming the downy hairs of the skin. If this change has not taken place over the whole skin before birth, it is completed during the first few months of the child's life. At birth or soon after, hairs usually grow actively on the scalp. At puberty, hairs appear in certain regions, such as the beard, axilla, and about the genitalia. In middle life and later, hairs, or vibrissae, grow about the nostrils and ears. In females, after the menopause, there is often an increase of pigmented and coarse hairs on the face, especially about the lips.

During life, the hairs have a limited existence, but they are not shed periodically over the entire body like the hairs of other animals. The hairs of different parts of the body have cycles of varying lengths.

Danforth[24] found that the period of growth in terminal hairs of the ears and eyebrows was about eight weeks and that the period of rest lasted three months. Bulliard[25] found a rest period of ten weeks for the hairs of the dorsum of the hand and a growth period of seven weeks. The hairs of the leg remain in a quiescent state for a period considerably longer than the growth period while, in the hairs of the axillary and pubic region, the reverse condition is found. In the pubic region, the period of rest is shorter than the growth.[26] In the scalp, the hairs have been said to have an existence of from 2–4 years, when they are shed and new ones are formed. How long the hairs generally grow and rest has not been recorded. For a detailed account of the growth in the human, one may turn to the extensive reviews of Hoepke[27] and Pincus.[28]

Puppy hairs and, in the case of the human, lanugo hairs, are produced first by follicles. Then the follicles produce larger, stiffer hairs. The follicle is thus able to produce hairs of different quality, but the means is unknown. Fine hairs of lanugo quality are often present in baldness. Many of them, in all probability, occupy follicles where coarse hairs once grew. What occurred to reverse the situation, or whether the follicle can be stimulated to form a coarse hair again, is of great interest to all of us.

Equally interesting are what controls the proliferative activity of the cells at the base of the resting club-hair and to what extent these cells retain their growth potentialities. By underfeeding experiments in the rat,[29] it was shown that the proliferative activity remained after long periods of quiescence. In hirsutism, the follicle retains its ability to produce a hair of a different quality after many years. Is failure of replacement in the human due to the loss of growth potentialities in the hair germ, to inhibition, to lack of stimulation, or to the lack of nutrition or is there an inductive action in the connective tissue which is lost?

Bibliography

1. Fraser, D. A. 1928. The development of the skin of the back of the albino rat until the eruption of the first hairs. Anat. Rec. **38:** 203–223.
2. Butcher, E. O. 1934. The hair cycles in the albino rat. Anat. Rec. **61:** 5–19.
3. Taylor, A. C. 1949. Survival of rat skin and changes in hair pigmentation following freezing. J. Exp. Zool. **110:** 77–112.
4. Dry, F. W. 1926. The coat of the mouse. Jour. Gen. **16:** 288–340.
5. Butcher, E. O. 1945. Pigmentation of hair on transplanted skin in hooded rats. Arch. Derm. and Syph. **52:** 347–350.
6. Butcher, E. O. 1937. Hair growth in adrenalectomized and adrenalectomized thyroxin-treated rats. Am. J. Phys. **120:** 427–434.
7. Butcher, E. O. 1940. The effects of irritants and thyroxin on hair growth in albino rats. Am. J. Phys. **129:** 553–559.
8. Ralli, E. P. & I. Graef. 1943. Stimulating effect of adrenalectomy on hair growth and melanin deposition in black rats, fed diets adequate and deficient in the filtrate factors of vitamin B^1. Endocrin. **32:** 1–12.
9. Whitaker, W. L. & B. L. Baker. 1948. Inhibition of hair growth by the percutaneous application of certain adrenal cortical preparations. Science **108:** 207–209.
10. Butcher, E. O. 1950. Unpublished data.
11. Butcher, E. O. 1936. Hair growth on skin transplants in the immature albino rat. Anat. Rec. **64:** 161–170.

12. Johnson, P. L., E. O. Butcher, & G. Bevelander. 1945. The distribution of alkaline phosphatase in the cyclic growth of the rat hair follicle. Anat. Rec. **93:** 355–361.
13. Johnson, P. L. & G. Bevelander. 1946. Glycogen and phosphatase in the developing hair. Anat. Rec. **95:** 193–200.
14. Bissonnette, T. H. & E. E. Bailey. 1944. Experimental modification and control of molts and changes of coat color in weasels by controlled lighting. Ann. N. Y. Acad. Sci. **45:** 221–260.
15. Rothschild, M. 1942. Changes of pilage in the stoat (*Mustela erminea* L.). Nature (London) **149:** 78.
16. Lyman, C. P. 1942. Control of coat color in the varying hare by daily illumination. Proc. New England Zool. Club **19:** 75–78.
17. Bissonnette, T. H. 1935. Relations of hair cycles in ferrets to changes in the anterior hypophysis and to light cycles. Anat. Rec. **63:** 159–168.
18. Basset, C. F., O. P. Pearson, & F. Wilke. 1944. The effect of artificially increased length of day on molt, fur growth, and priming of silver fox pelts. J. Exp. Zool. **96:** 77–83.
19. Segall, A. 1918. Ueber die Entwicklung und den Wechsel der Haare beim Meerschweinchen (*Cavia Cobaya* Schreb). Arch. f. Mikro. Anat. Bd. **91** (Abt. 1): 218–291.
20. Hofer, H. 1914. Das Haare der Katze, seine Gruppenstetlung und die Entwicklung der Beihaare. Arch. f. Mikro. Anat. Bd. **85** (Abt. 1): 220–278.
21. Dawson, H. L. 1930. A study of hair growth in the guinea pig (*Cavia cobaya*). Am. J. Anat. **45:** 461.
22. Macleod, J. M. H. & I. Muende. 1946. Pathology of the Skin: 222. Paul B. Hoeber. New York.
23. Trotter, M. 1932. The hair. Special Cytology, by Cowdry, E. V. 2nd ed. **1:** 41. Paul B. Hoeber. New York.
24. Danforth, C. H. 1925. Hair with special reference to hypertrichosis. Chicago, A. M. A.
25. Bulliard, H. 1923. Influence de la section, et du rasage répeté sur l'evolution du poil. Ann. de dermat. et syph.: **4:** 386.
26. Trotter, M. 1924. The life cycles of hair in selected regions of the body. Am. J. Phys. Anthrop. **7:** 427.
27. Hoepke, H. 1927. Die Haut, Handb. d. mikr. Anat. (U. Möllendorff, 3) pt. 1, Berlin.
28. Pincus, F. 1927. Die normale Anatomie der Haut, Handb. d. Haut. u. Geschlechtskrankheiten (1): 77.
29. Butcher, E. O. 1939. Hair growth in young albino rats in relation to body size and quantity of food. J. Nutr. **17:** 151–159.

THE HAIR CYCLE OF THE MOUSE AND ITS IMPORTANCE IN THE STUDY OF SEQUENCES OF EXPERIMENTAL CARCINOGENESIS

By S. Burt Wolbach
Director, Division of Nutritional Research, The Children's Hospital, Boston, Massachusetts

This paper is the outcome of experiments made in 1937, 1938, and 1939 for the purpose of following sequences antecedent to tumor formations resulting from applications of 3,4-benzpyrene and 20-methylcholanthrene to the skin of mice. My purpose is to present observations which I believe are of value for the interpretation and evaluation of similar published studies, many of which, fortunately for such purposes, are well illustrated by photomicrographs. I have seen very few that have not been objectively described previously.

For my own understanding, I was forced into a study of the hair cycle of the mouse and found that consideration of the whole pattern was important, from the initiation of new follicle formation, resulting in a structure extending from the epidermis to the muscle panniculus, to the ascent of the completed hair, where its club end comes to rest close to the surface of the skin. Growth and atrophy are involved, and a knowledge of both sequences is essential for the interpretation of the pathologic results with which we are concerned. No feature of the microscopic pictures found in chemically treated skins can be regarded as static. I make no attempt to include cytological descriptions. I have excluded, as far as possible, complications resulting from trauma by companion mice, and from the presence of ectoparasites. Protection of areas by crust formation has also been considered.

The Hair of the Mouse. Hair is the product of epithelium. The initial growth originates in buds composed of cells of the basal layer of the epidermis. Concurrently, a cluster of mesenchymal cells, the future papilla, may be seen. Which structure, epithelial or mesenchymal, is formed first, has not been determined. Stöhr,[1] for human lanugo hair, gives lengthy consideration to this problem.

The development of mouse hair after birth is rapid. At the age of one day, in the first follicles to form, the papilla cells have invaginated the hair bulb, the hair matrix is cone-shaped, and the hair shaft is forming. At four days, the hair bulb has reached the subcutaneous muscle panniculus and the tips of the hair have penetrated the epidermis. At seven days, the internal sheath cells (layers of Huxley and Henle) have disintegrated and the hair canal has formed. At 11 to 14 days, growth of hair is about completed, the hair club has begun to form, and the cells of the matrix have begun to atrophy. At 22 to 23 days, the hair club has reached its final position in the dermis. Growth of the follicles of the first hair coat does not begin simultaneously. The larger covering hairs take the lead. According to Gibbs,[2] in the first five days, there are four major waves of hair follicle development, but new follicles continue to form on the 6th, 7th, and

8th days. My own less careful study of mouse skins at 2, 3, and 4 days of age supports Gibbs's observations. I have evidence that this initial order of follicle growth in crops is maintained to some extent in the periodic formation of new follicles that continues throughout the life of the mouse and is well under way by the 28th day. At the age of 28 days, the second growth of follicles has reached a stage corresponding to that of follicles in mice 3 to 4 days old; at 36 days, hair canals have formed. The period of rest of the follicle, *i.e.*, quiescence, must therefore be very short in the mouse.

Oyama[3] has described and illustrated in detail the growth of the first generation of the mouse hair, from the appearance of the "hair germ" to the completion of growth. He did not describe the ascent of the hair club to its final position. Dry,[4] for the mouse, states that about one month is required for the completion of growth of the first generation of hair, that the second generation is completed at about two months, and that the third generation is completed at about three months, though often delayed. Dry noted that hairs are not shed before completion of the second generation, but up to 4 hairs may be retained in one follicle. These multiple-haired follicles should, in my opinion, be called "compound follicles." Dry also showed that the initiation of follicle formation of succeeding generations of hairs does not take place simultaneously over the whole body but proceeds in orderly waves of definite pattern.

Butcher[5] has described the growth of hair of albino rats beyond the age of 5 days. According to him, the rate is slower than in the mouse. He found that growth was completed at about 17 days of age, and, at 22 days, the hair club had ascended to its final position. A quiescent period of about 10 days followed, after which a new off-shoot of follicle formation appeared. At 53 days, the quiescent period of the second hair was reached. He noted that the new hair did not push out the old hair but, "in many instances," remained beside it. He also found that the growth of hair follicles on the ventral surface of the rat began, and was completed, 4 to 5 days in advance of similar events on the dorsal surface.

Satisfactory evidence that the periodic formation of new hairs in the mouse was not uniformly timed over the surface of the body was given by Dry[4]. He described and charted the order of appearance and completion of growth of the first generation of the mouse's hair as follows: on the front of the head at the margin of the snout areole, at the root of the tail, on a band lateral and posterior to the ears, and on the fore- and hindlegs. The development of hair then spread back from the front of the head and then upon the abdomen. He followed and charted the order of the appearance and development of the second and third generation of hair, somewhat different from that of the first generation, but bilaterally symmetrical. He gave references to earlier publications by other workers, indicating that, in general, the features in hair succession, by waves of growth, is common to other mammals. Dry's paper should be consulted by workers interested in this field.

Indications that the periodic growth of hair followed a definite pattern in normal rats were given by Butcher[5] and Emmens,[6] and in the recovery of rats nurtured upon deficient diets, which followed restoration of the missing element, by Unna *et al.*[7], as shown by the illustrations for pantothenic acid, and by Cunha *et al.*[8], for inositol. The latter stated that, in inositol deficiency, "The hair loss starts in the dorsal part of the head and proceeds bilaterally along the sides to the tail region and then downward to the hind legs. With inositol therapy the hair returns inversely to the loss, proceeding from the caudal portions forward."

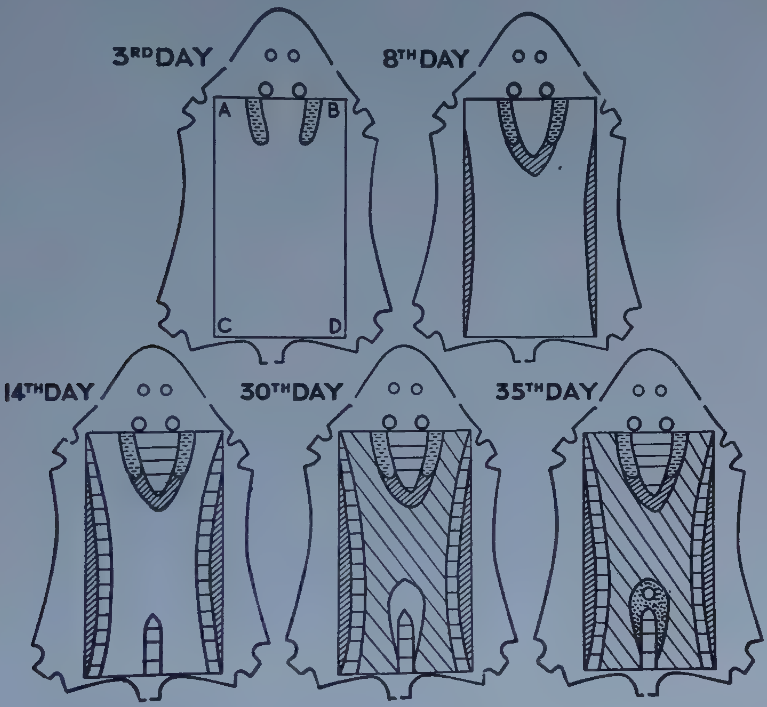
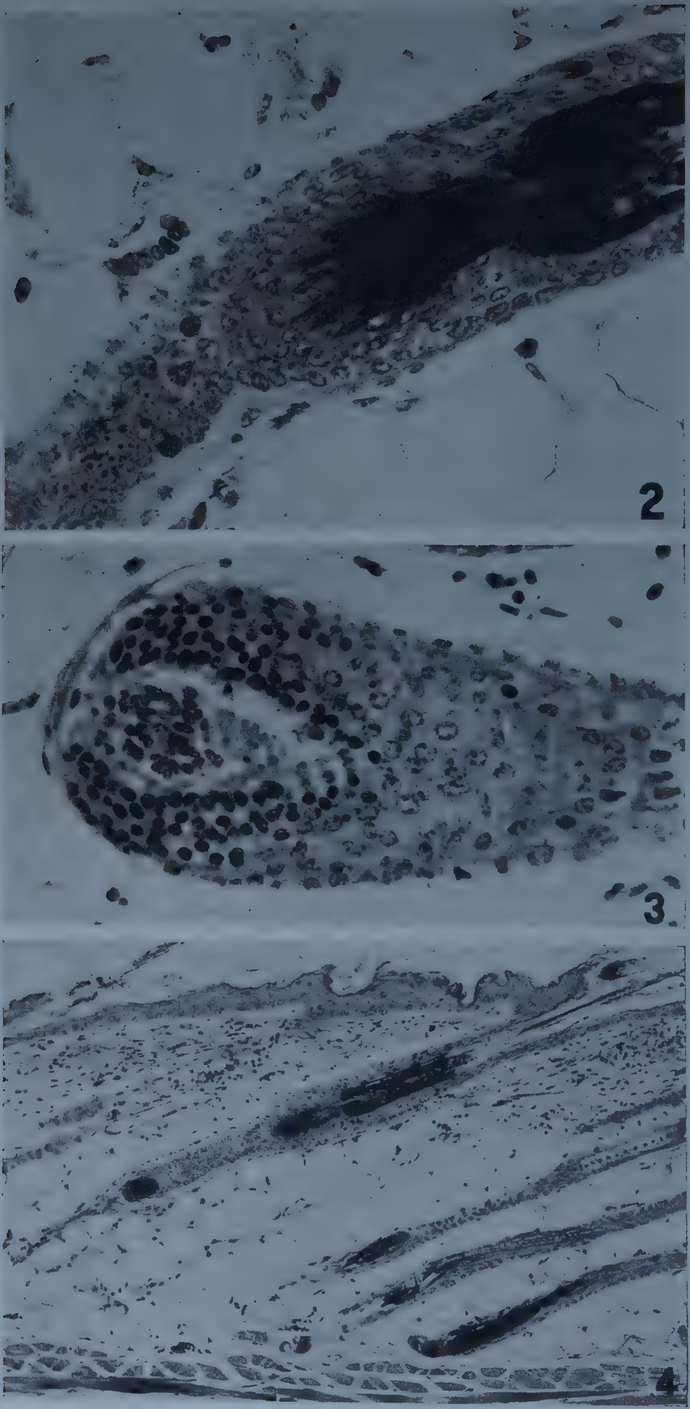


FIGURE 1. Regeneration of hair at 3, 8, 30, and 35 days after shearing the area of A, B, C, D. Similarly hatched areas are those in which the hair is growing at the same time and in which the alloxazine pigmentation would develop in response to administration of the compound at about that time. (Reproduced with the kind permissions of Professor Haddow and the Editor of Nature.)

The most complete information regarding the orderly pattern of periodic new hair growth for the rat was reported by Haddow *et al.*[9, 10] They found that injection of a flavin (9-phenyl-5: 6 benzo-iso-alloxazine) produced an orange-yellow coloration of hair in process of regeneration. The color pattern which resulted demonstrated that new follicle formation follows a definite pattern, consisting of regular waves symmetrically distributed about the longitudinal axis, which is a "continuous rhythmic process." The results of the injection of the chemical were confirmed by observing the order of appearance of hair, following shaving of the dorsal and lateral sides of rats (FIGURE 1). Fragmentary observations of my own and the facts presented by Dry[4] indicate strongly that the pattern of periodic new hair formation of the mouse is closely similar, if not identical, to that of the rat.

An important consequence of the occurrence of the periodic new forma-



FIGURES 2, 3, and 4 (*see facing page*).

tion of hair in waves is that it is possible in a single section of mouse skin, prepared for microscopic study, to find 2 or more stages of follicle development.

Our knowledge of two sequences of hair growth in the mouse is fairly complete; one, the growth cycle of individual hairs, the other, the wave-like pattern, as the periodic new formation proceeds. Both must be kept in mind in the study of the effects of applications of chemical agents, carcinogenic or otherwise, to the skin of mice, or of any other mammal. Before discussing these sequences in relation to experimental carcinogenesis, I wish to present the results of my own efforts at elucidation of two details of the cycle of hair growth, which have been the subject of some differences of opinion. These are the fate of the cells of the papilla and the mechanism of ascent of the hair club from its site of formation deep in the subcutaneous fat to its final site in the corium. Both details have application in the interpretation of results following applications of "carcinogenic" chemicals.

The development of the hair follicle and the details of formation of the hair are precisely similar processes in man and other mammals studied and are too well known to require recounting.

As the growth of the hair ceases, the multiplication of cells of the matrix ends, and cells above the apex of the papilla become converted into cornified spindles continuous with the hair cortex. These cornified spindles, because they arise at the apex of a cone, are convergent. They interdigitate with surviving cells derived from the matrix which are, or become, continuous with external sheath cells. These convergent, cornified, spindle-shaped cells form the hair club and serve to anchor the hair (FIGURE 2). At cessation of growth of the hair, while the hair club is being formed, the hair canal has been formed by the disintegration and disappearance of the internal sheath cells. Concurrently, most of the cells of the matrix atrophy, but not before there has been multiplication of the deepest cells of the bulb surrounding the base of the papilla. The product of this last proliferative activity contributes to a column of cells of matrix origin, the hair stem ("haar stengel" of German authors), which later lies between the hair club and surviving cells of the papilla. The hair stem becomes surrounded by the glassy or vitreous membrane. Concurrently with the atrophy of the matrix cells, most of the papilla cells atrophy, but others survive, divide, and give rise to a group of deeply basic staining concentrically arranged cells (FIGURE 3), which I shall call the papilla rest.

Up to this period, the external sheath has been composed of relatively large cells, particularly from the level of the hair club formation to the enlargement of the follicle, deep to the sebaceous gland (known as the "Wulst" in German literature). The cells of the external sheath atrophy and, con-

---

FIGURE 2 (*see opposite page*). To show the hair club and the anchoring spindles of cornified cortical cells. Ascent of this hair is not yet completed. Note the glassy membrane and upper end of the hair stem. (320 dia.)

FIGURE 3. To show atrophy of matrix cells and the newly formed rest of papilla cells. Glassy membrane formation is just perceptible. (320 dia.)

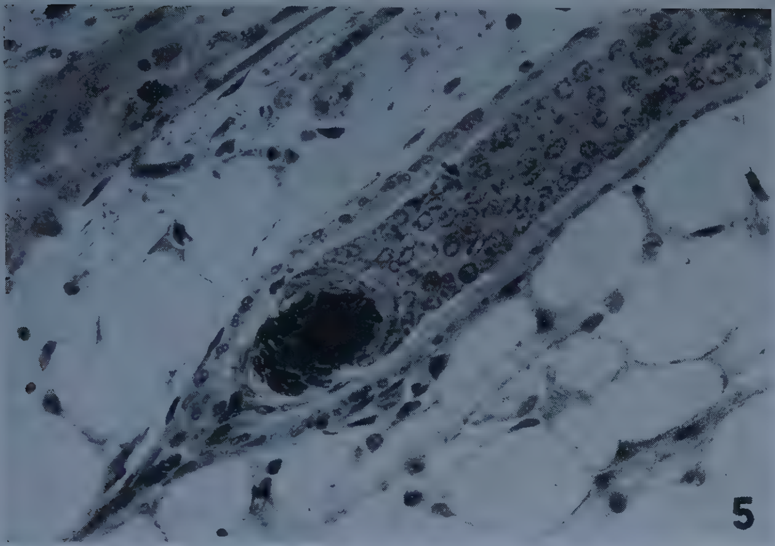
FIGURE 4. To show a cover hair in about the middle period of ascent. The glassy membrane, hair stem and papilla rest are conspicuous structures. The hair canal is formed, though some of the deep internal sheath cells are retained, probably the result of one application of methylcholanthrene sixty hours previously. Note effect on three other follicles caught earlier, before ascent had begun. (180 dia.)

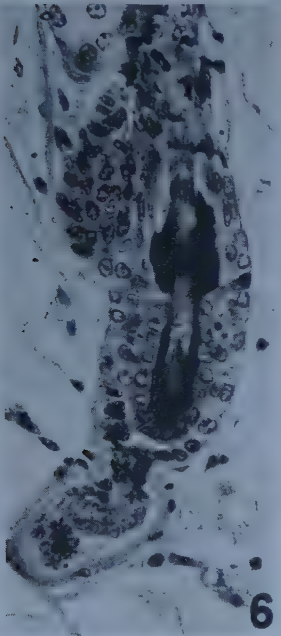
currently, there is a very rapid formation of collagen by the connective tissue sheath, most abundantly below the level of the hair club (FIGURES 4 and 5). The layer of collagen constitutes the glassy or vitreous membrane. It apparently compresses the atrophying cells of the matrix and external sheath. Its formation is probably induced by the atrophy of these cells, because, similarly, formations occur in the human body following rapid atrophy of epithelial structures, notably the great thickening of the tunica propria of the seminiferous tubules of the testes, following atrophy of the epithelium, resulting from diverse causes.[11] In the involution of the mammary gland after lactation, a similar rapid deposition of collagen occurs.

Dry[4] doubted the connective tissue origin of the glassy membranes. Stöhr[1] regarded the glassy membrane to be of connective tissue origin, but also described, in man, an inner layer of external sheath origin, probably, in my opinion, a cuticular border of epithelial origin. Segall[12] described, for the guinea pig, sequences precisely similar to those I have outlined above for the mouse. The glassy membrane, he stated, is of connective tissue origin and, by its contraction, it plays a part in the ascent of the hair club: it tends to squeeze the follicle upward. Segall also noted the formation of a cluster of deeply-staining cells in the papilla and its separation by the hair stem from the hair club. This cell cluster, he asserted, becomes the papilla in the succeeding follicle formation. Dry,[4] for the mouse, was vague in regard to the origin of the papilla rest cells and referred to them as the hair germ "destined to give rise to the companion hair of the next generation," and presumably regarded them as of epithelial origin. Segall[12] discussed at considerable length the mechanism of the ascent of the hair, without coming to a definite conclusion of his own. He stated that the explanation would be found in the thickening and contraction of the glassy membrane.

My own observations confirm, in part, those of Segall. The glassy membrane takes stains used for demonstrating collagen. After fixation in Zenker's solution and staining with Mallory's acid fuchsin-aniline blue connective tissue stain, the glassy membrane takes a deep blue coloration. It is separated from the connective tissue corium by a row, arranged in parallel, of connective tissue cells. The collagen fibers of the glassy membrane, when first formed, are straight, and parallel the long axis of the hair stem. The papilla rest, by the time of development of the glassy membrane, has been expressed from the hair bulb and lies deep to the hair-stem epithelium but enclosed by the glassy membrane. As atrophy and disappearance of the epithelial cells of the hair stem proceeds, the glassy membrane becomes more and more corrugated and, by this process, the papilla rest is brought up into close apposition to the base of the hair follicle, at a time when the hair club has almost reached its final position (FIGURE 6). All the sequences just related follow the disappearance of the internal sheath cells, the formation of the hair canal and hair club, and the arrangement of cells enclosing the hair club.

It is possible that the hair stem to which the final proliferative activities





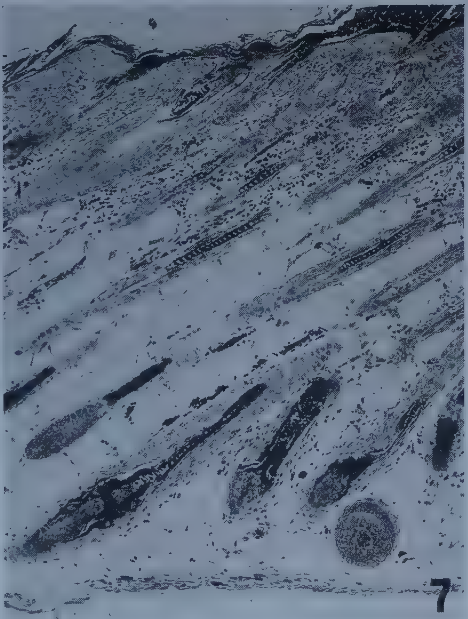


FIGURE 5. Higher power (310 dia.) of cover hair in FIGURE 4. Nuclei of cell in the hair stem appear to be viable. The row of cells which are the source of the glassy membrane collagen are well shown. Note enclosure of the densely stained papilla rest by the glassy membrane.

FIGURE 6. To show a hair at near completion of ascent. Note corrugation of an already thinned glassy membrane, atrophic remains of stem cells, and the papilla rest. (320 dia.)

FIGURE 7. To show the 24-hour effect of a single application of 0.3 per cent methylcholanthrene solution after hair canals have formed. Note the degenerating matrix in each of three hair bulbs. (120 dia.)

of the hair bulb contribute, as a result of compression by the glassy membrane, may be the source of an upward impulse to the hair. If so, the duration must be very brief, because advanced atrophy of the cells and extensive corrugation of the glassy membrane occurs before the ascent of the hair is completed. The completed hair lies free in the hair canal and offers no resistance as the anchored club end is drawn upward by the contracture of the external sheath. The important factor in the ascent of the hair is reduction in length of the external sheath resulting from diminution in size and number of its cells. The corrugation and contraction of the glassy membrane bring the papilla rest into its final position in relation to the resting follicle. Even as the glassy membrane undergoes these changes, it diminishes in thickness. It disappears as the growth cycle of the follicle is completed. Its disappearance, the disappearance of a conspicuous collagenous structure, should be placed in the same category of normal phenomena as the disappearance of the collagen of the breast as the mammary gland develops in pregnancy and the changes in the symphysis pubis during late pregnancy.

In the mouse, the resting or quiescent period in the follicle is very short, at most a matter of a few days. I have found that, in mice 28 days old, the second generation of follicles was well advanced, comparable to 3 or 4 days' postnatal growth of the first generation. This would indicate a shorter period for growth of the first two generations than one month each, as given by Day.[4]

The papilla rest descends with the growth of the new follicle, and from it the new papilla forms. This sequence is repeated with the growth of each generation of hair. In the mouse, no blood vessels enter the papilla. Capillaries are found immediately below its base. There have been differences of opinions regarding the persistence of the papilla cells throughout the succession of generations of hair. Segall[12] has reviewed the subject. His account of papilla sequences of guinea pig hair successions is in accord with mine for the mouse. Dry,[4] for the mouse, made no general statement on this question, but did state, "In the mouse, in the follicles which produce vibrissae, I have come to the conclusion that another vibrissa begins to be formed as soon as the growth of its predecessors is completed, and that the original papilla persists."

Throughout the life of the mouse, the successions of generations of hairs require rates of proliferation and growth of epithelial cells, apparently far greater than those of the epidermis. Glücksmann,[13] using data relating to mitotic activities, obtained by other workers and through his own studies, calculated that the life span of an epidermal cell, from mitosis to desquamation, was approximately 21 days: "This period is of the same order as that of the hair cycle of the mouse (3 to 4 weeks)." If we compare the few layers of cells of the mouse epidermis with the periodic new production of cells in hair formation, we must conclude, while awaiting other factual data, that hair-follicle epithelium is normally by far the most active of epidermal tissues to which carcinogenic chemicals have access.

The Hair of the Mouse Subjected to Applications of 3,4-Benzpyrene and

20-Methylcholanthrene.[*] 3,4-Benzpyrene was used in 0.15 and 0.3 per cent strengths in benzene solution, 20-methylcholanthrene in 0.3 per cent benzene solution only. Commercial albino mice, males and females in equal numbers, were used. Purchased at 6 to 10 weeks of age, they were kept under observation for a 2–4 weeks' period, to exclude disease, before beginning the experiments. The applications were made, after clipping the hair with an electric razor, on the back, in an area about 3 cm. square in size, centering over the midscapular region. The 0.3 per cent solutions were applied twice a week; the 0.15 per cent solutions, three times weekly. Approximately 0.0375 mg. of the chemical in 0.15 per cent solution was deposited at each application; for the 0.3 per cent solution, approximately 0.075 mg.

More than 1000 mice were used. The effects of single applications were followed, after periods ranging from 5 minutes to 30 days. In serial studies with benzpyrene, groups of mice receiving from 2 to 54 applications over periods of 1 to 21 weeks were followed. With the 0.15 per cent solution, some mice were set aside for recovery periods of from 2 to 8 weeks as follows: at 15, 20, 30, 40, 47, 48, and 54 applications. With the 0.3 per cent solution, recovery periods of from 1 to 6 weeks were begun after 9 applications as follows: at 11, 15, 22, 24, 30, 34, 35, 39, and 40 applications. In the methylcholanthrene series, recovery periods of from 2 to 20 weeks were begun as follows: at 6, 12, 18, 24, 30, 36, 42, and 48 applications.

Skin and other tissues selected for study were fixed in Zenker's fixative and embedded in paraffin. From each block, 6 to 12 sections in series were mounted upon slides, so that in each such preparation of skin there were many follicles completely covered by serial sections. Care was taken to have the plane of sectioning as nearly parallel as possible with the direction of slope of the hair. The stains employed were Mallory's eosin-methylene blue, Mallory's acid fuchsin, aniline blue connective tissue stain, and Weigert's elastic tissue stain.

It does not come within the scope of this paper to record the complete study of this whole number of mice. Brief mention will first be made of events following cessation of applications. The literature pertaining to skin carcinogenesis in animals contains many discussions concerning the question of whether the chemicals used have a specific carcinogenic effect upon the epidermis and hair follicles. This question has been answered. The results of my experiments have led me to the conclusion that most, if not all, changes described as precancerous are reversible.

After as many as 18 applications of 0.3 per cent methylcholanthrene in 6 weeks, and 30 applications of benzpyrene in 11 weeks, after 19 and 8 weeks' recovery periods respectively, the only microscopic evidence in some mice of previous damage and papilloma formations was a decrease in numbers of hairs and increased density of the corium. Short duration experiments, in the endeavor to find distinctive cytological details, have produced nothing of importance to offset the results of recovery experiments. Pullinger[14] has given a good description of the early changes produced in the epidermis by various carcinogenic hydrocarbons. Cowdry[15] has written an excellent review of the subject. He stated, "Neither in the literature . . . nor in our observations is there convincing evidence that a single change or multiple changes actually and directly cause malignant transformation." Howes[16] gave as one of his conclusions to experimentation with methylcholanthrene that, "No justification can be found for the theory that the carcinogen stimulates the cells directly to the formation of a tumor." In my own work, tumors, benign or malignant, which appeared during the recovery periods, if sufficient time were allowed to elapse before killing the animals, were almost always surrounded by skin with normal epidermis and hair follicles, though usually with a denser than normal corium.

---

[*] The experimental work done in 1937 to 1939 was planned with the assistance and advice of Dr. M. T. Shear, now Chief of the Chemotherapy Section of the National Institutes of Health. He supplied the mice and chemicals and released the services of a trained assistant, Dr. Israel Kapnick, who made the applications and recorded gross changes. To Dr. Kapnick, then a medical student, the author acknowledges indebtedness.

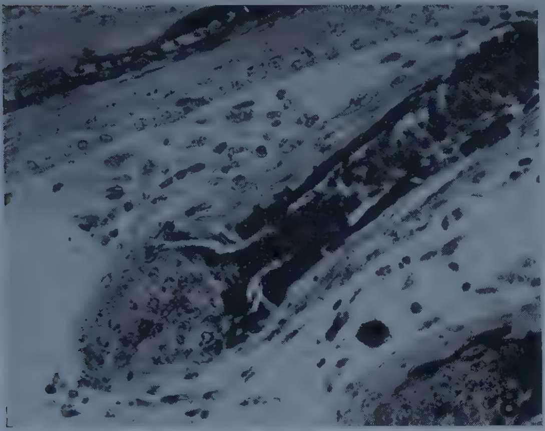
Persons interested in the problems presented by the fact that tumors arise focally after diffuse treatment of the skin may find some measure of explanation in what I describe below. They will find much of interest in the excellent monograph by Guldberg,[16] who used tar on mice as the agent, and in the papers by Rous and associates, who also used tar upon rabbits.[17–20]

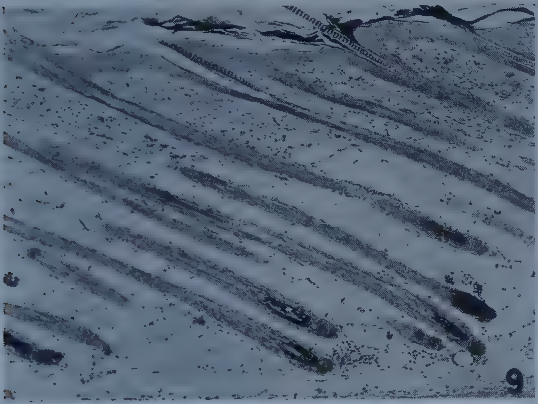
Very few authors of papers on experimental skin carcinogenesis have given much attention to hair follicles. In fact, a number have recorded normal sequences of growth as evidence of hyperplasia. Others have regarded the presence of compound follicles, those with two or more hairs, as evidence of increased hair formation induced by the chemical agent. Glücksmann,[13] Guldberg,[16] and the Silberbergs[21] gave more than casual attention to hair follicles. None of them correlated effects of applications with the various stages in the hair follicle cycle. The Silberbergs, who used guinea pigs (which do not give rise to tumors), concluded that new hair follicles from the epidermis were formed, and that the hair growth in the flank was more responsive than that in the ear. No reference was made to normal periodic new-follicle formation or to the wave-like progression of this process, either in the experimental animals or controls. Obviously, in two guinea pigs of different litters, the hair of the same region may be in different stages of development so that their "normal controls" were, in reality, not controls.

In the mouse, new follicles do not form from the epidermis, under any circumstances, after the original postnatal formation has been completed.

Because of the wave-like progression of the periodic hair follicle cycle, it is impossible to know, by gross observation, the stage of hair formation in the area of first application of the chemical. I have often found in normal mice, or mice sacrificed soon after one application, two and even three stages in follicle formation in the 2 cm. long strip, centering in the midscapular region, taken for study. Often, in one end, the follicles would be at rest, while at the opposite end they would be approaching completion of growth, in the early stage of hair canal formation.

Obviously, applications of solutions to hair follicles, when the hair canal has been formed, reach depths, and come into contact with structures not possible in the quiescent follicle. A single application of 0.3 per cent 20-





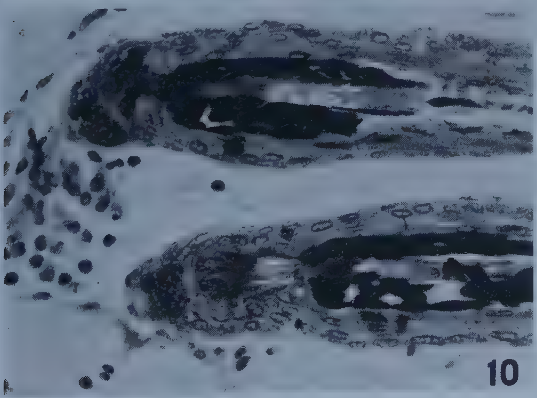


Figure 8. High power of the central hair bulb in figure 7. Note the stratified squamous epithelium of the external sheath and the degeneration of apex of the matrix. (500 dia.)

Figure 9. Stalled hairs, following repeated applications of 0.15 per cent benzpyrene solution. Note that hair clubs have formed deep in subcutaneous fat. (120 dia.)

Figure 10. High power (310 dia.) of two stalled follicles shown in figure 9. Note stratified squamous epithelium of the external sheath, the completed growth of the hairs, with club formation and the papilla rests in apposition to the quiescent follicles. As a result of the chemicals, one hair club is imperfectly formed.

methylcholanthrene to a follicle with a newly formed hair canal can result in partial or complete killing of the hair matrix, and conversion of the external hair sheath into a keratinizing squamous epithelium. It requires about 24 hours for these changes to become fully developed (FIGURES 7 and 8). The reaction is precisely similar to that seen at the same period in the epidermis, and which has often, for later periods, been described as hyperplasia with hyperkeratinization. In other words, the agent produces injury which is followed by reparative sequences familiar to pathologists, the extent of which corresponds with the degree and duration of damage. Killing of cells, whether of the epidermis or of external sheath cells, is followed by reparative proliferation and, in the latter structure, as is the case with many other nonkeratinizing epitheliums, conversion into a stratified keratinizing epithelium.

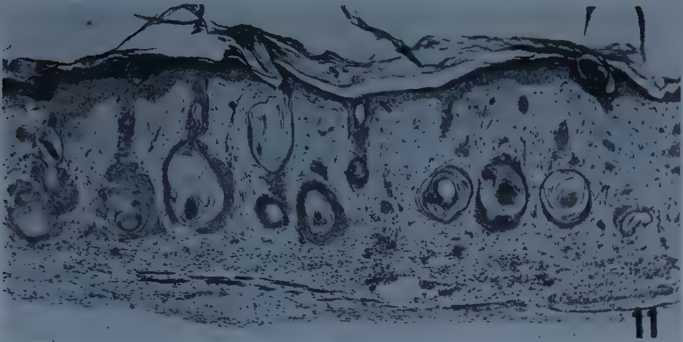
The result of the keratinizing changes of the external sheath epithelium is to prevent ascent of the hair. This causes the hair club to remain at its site of formation. The hair stem and glassy membrane are incompletely formed, and their sequences are difficult to follow. Such hairs I have designated stalled hairs (FIGURES 9 and 10). The result of repeated applications to such hairs, in many instances, is complete destruction of the matrix and papilla in the succeeding follicle cycle, owing to the fact that the hair canal of the stalled hair remains open. If the outermost cells of the bulb survive, a cyst lined with keratinizing epithelium results. The epithelial lining of such cysts usually atrophy synchronously (a cyclic response) with the atrophy of the matrix of normal hair follicles in the same zone of periodic cyclic progress. The end result is a collection of giant cells formed about keratinized cells and remains of the hair club.

In skins subjected to repeated applications of methylcholanthrene over many weeks, during which follicles of several generations have been caught in the stage of open hair canals, it is possible to find as many as three stages of such cysts, recently formed ones, collections of giant cells of recent origin, and, oldest of all, collections of giant cells reduced in size and undergoing atrophy. At any stage of the ascent of the hair, while the hair canal is patent, destruction of the matrix is possible and cysts at various depths may result, or keratinizing enlargements of the follicle near the surface may form (FIGURES 11 and 12). Both these results have frequently been interpreted in the literature as downgrowths of the epidermis with cyst formation.

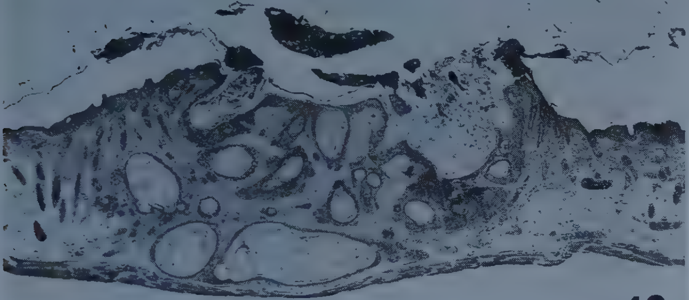
The contrast in results is great when the applications are begun while the follicle is quiescent. The solution then can come in contact with only relatively inactive cells in which the hair club is embedded and by which the shaft is surrounded.* The effect upon the resting follicle is like that upon the epidermis. There is reparative proliferation resulting in increased keratinization and consequent depilation (FIGURE 13). If, as is usually the case in regions with quiescent follicles at the beginning of the applications, the papilla rests are not destroyed, new follicle formation begins as per

---

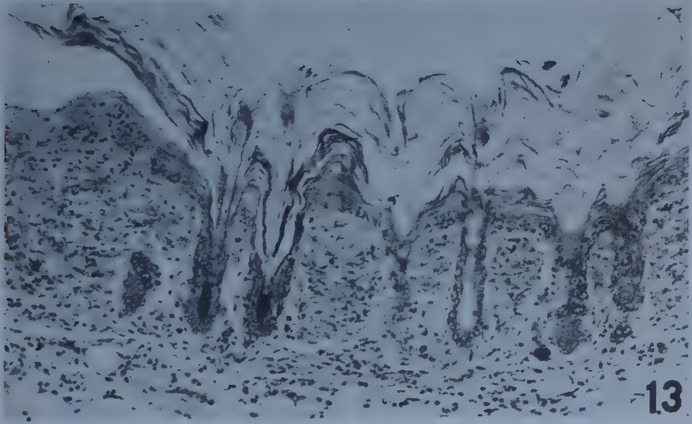
* The secreting sebaceous gland cells are destroyed but the basal cells survive and, in spite of continued repetition of applications, may regenerate and form normal-appearing glands. More often, they do not reconstruct the gland until applications have ceased to be applied. Arrector pili muscle fibres remain indefinitely with little change, unless severe inflammatory reactions arise in the corium.



11



12

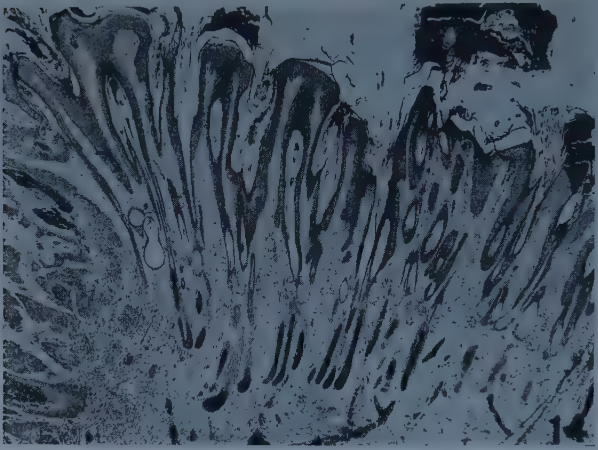


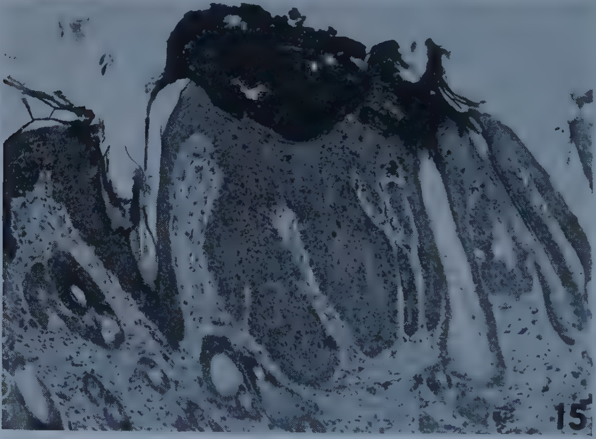
13

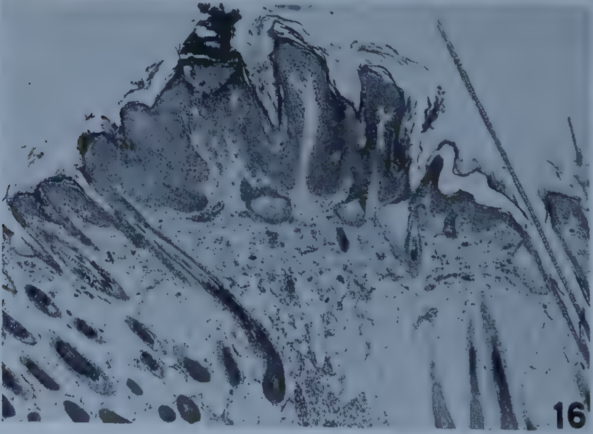
FIGURE 11. Cysts lined with stratified keratinizing epithelium, resulting from seven applications of 0.3 per cent methylcholanthrene solution in fifteen days. Because of the level of these cysts, the first applications must have been made early in the ascent of the hair. (120 dia.)

FIGURE 12. Cyst formations at various levels resulting from forty-eight applications of 0.3 per cent benzpyrene solution during sixteen weeks. The mouse was killed after a recovery period of twenty-one days. Processes indicative of early tumor formations are arising from some of the cyst walls. (28 dia.)

FIGURE 13. To show the result of three applications of 0.3 per cent benzpyrene solution during ten days to quiescent hair follicles. Depilation has not yet occurred, but the desquamation of keratinized cells, which leads to depilation, is in progress. Note other changes, including hyperkeratinization of epidermis and infiltration of the corium. (200 dia.)







Figures 14, 15, and 16 (*see facing page*).

schedule for that region. I have found no evidence that depilation, by means of the agents used, hastens new follicle formation. Our own gross observations of return of hair and numerous descriptions found in the literature indicate that the time required for new hairs to make their appearance (grossly) varies in regions of the same depilated area. Furthermore, the order of appearance follows a wave pattern. These facts indicate what the microscope proved: that, while depilation of a quiescent hair has occurred, a new follicle formation from that follicle may have been in progress for one to ten days, or for the duration of any period preceding hair canal formation. Until the hair canal has formed, the down-growing follicle is not accessible to the solution. The rate of appearance of new hair is determined by the stage of new follicle formation and the time of depilation.

If the papilla cells are destroyed, no follicle formation takes place. As long as the papilla cells survive, in spite of many repeated applications and sclerosing changes in the corium, the periodic hair follicle cycle continues, as nearly as I can ascertain, according to schedule. The fact that the developing hair follicle can descend through a thickened and sclerosed corium presents a problem of interest in the category of similar normal sequences, shown in the disappearance of dense collagen of the breast, as the mammary gland develops in the first pregnancy.

Repeated applications, after hairs have become stalled, result in epiderminization of the hair canals down to the level of the hair club, at whatever depth this structure becomes fixed, *i.e.*, at any stage of its ascent. At this lower depth (FIGURES 9, 10, and 14), the periodic follicle cycle is continued and the sebaceous glands of succeeding follicle generations lie deep where the hair emerges into the tube of stratified squamous keratinizing epithelium. I have not been able to find arrector pili muscle fibers attached to those follicles, even after long recovery periods.

Papillomas develop from hair follicles which have lost their papilla rest cells (FIGURE 15). This conclusion was arrived at after failure to find these cells in any stage of papilloma formation, and with the knowledge that papilla cells may be destroyed by repeated applications, either following depilation at the quiescent stage, or in active follicles after the hair canal has been formed.

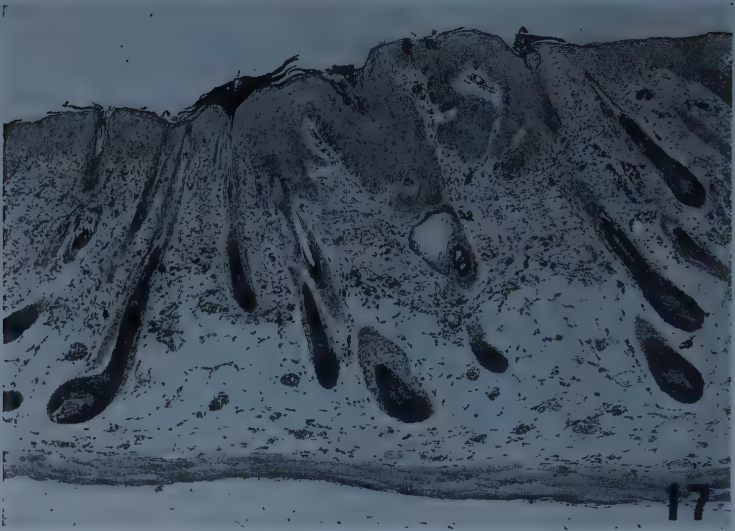
The follicle deprived of its papilla cells is no longer the source of new follicle formation. A cyclic proliferative response does occur, accompanied by growth of epidermis-like epithelium and new sebaceous gland formation (FIGURE 16). The first response to the unknown stimulus of cyclic hair regeneration is the earliest detectable stage in papilloma formation (FIG-

---

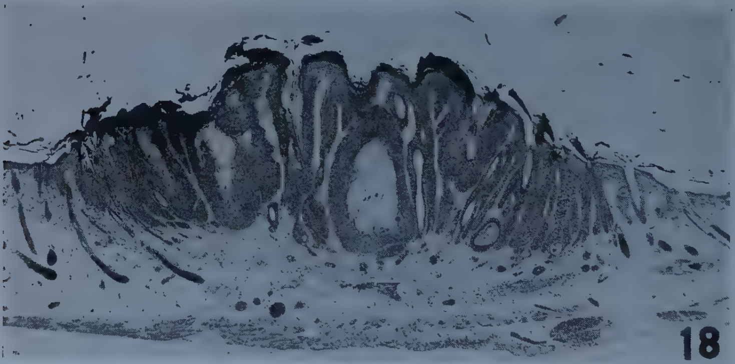
FIGURE 14 (*see opposite page*). To show periodic follicle formation at a deep level, resulting from stalling of hairs. From a mouse which received forty-one applications of 0.15 per cent benzpyrene solution during sixteen weeks, followed by a recovery period of sixty days. The follicles are adjacent to a small carcinoma. Note deep mouths of the follicles lined with stratified keratinizing epithelium and low sites of sebaceous glands (40 dia.)

FIGURE 15. An early stage in papilloma formation represented by two follicles which have lost their papilla cells, while in, or close to, the period of completed growth or quiescent stage. From a mouse which received thirty applications of 0.3 per cent benzpyrene solution during sixteen weeks. No recovery period. (165 dia.)

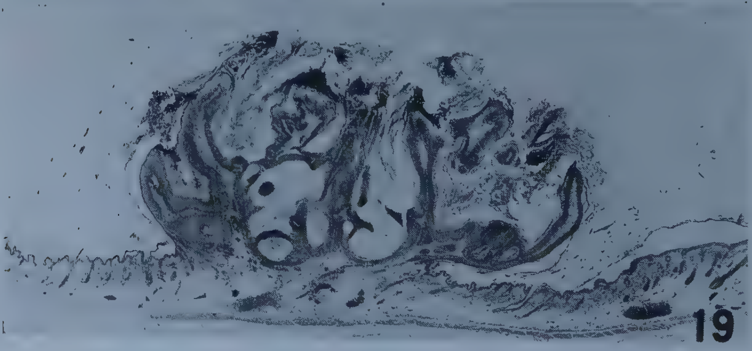
FIGURE 16. Early papilloma formation initiated from follicles which had lost their papilla cells while in, or close to, the quiescent period. Note adjacent active follicles. Sebaceous glands have formed from the epithelium of the developing papilloma. From the same mouse from which FIGURE 15 was made. (165 dia)



17



18



19

FIGURES 17, 18, and 19 (*see facing page*).

URES 15, 16, and 17). The resulting new formation, if the process begins low down from follicles of stalled hairs, is somewhat different from that which follows when initiated in follicles which were in quiescence.

From stalled follicles, deeply seated keratinizing cysts and tubes are early formations. If these structures continue to grow, the cysts finally communicate with the surface (FIGURES 18 and 19). Proliferation and growth of the cells lining cysts and tubes increase the surface area of the formation, resulting in overhanging of the structure with pedicle formation.

Usually, the cysts lined with keratinizing epithelium, described and often profusely illustrated in the literature of chemically induced tumors in mice and rabbits, have been interpreted as the result of downgrowth of hyperplastic epidermis.

Formations originating from non-stalled quiescent follicles (FIGURE 16), owing to increase in surface covering dimensions, project above the surface of the skin without cyst formation. I have not been able to find evidence of papilloma formation from the epidermis. Guldberg[16] also concluded, that, in mice treated with tar, papillomas arose from hair follicles. He wrote "Where the hair-sacs show a periodic hypertrophy of growth, it is very natural to suppose that the formations which develop from the hyperplastic hair-sacs may also, in many cases, show a periodic growth. This periodic growth of hair may be a contributory cause of the phenomenon that the papillomata, after having existed for a time, may again disappear, only to present themselves once more, after an interval corresponding to a period of hair growth."

While I have not been able to correlate the formation or disappearance of papillomas with the periodic hair cycle of the region, I have been able to correlate changes indicative of fluctuating activities of papillomas with those of the hair follicles of the region. During growth of the hair follicles, the cells of a papilloma in the same region are larger and more deeply basic staining (eosin-methylene blue), with more numerous mitotic figures than those of a papilloma in a region with atrophying follicles or with quiescent hairs.

In the consideration of papilloma formation, it is difficult to draw a line between the hyperplastic response to long-continued, or often repeated, injury and new growth formation. One aspect of this problem is the well known fact that most papillomas, and carcinomas arising from them, appear focally in relatively few numbers in areas treated with carcinogenic agents. This is suggestive of the introduction of an additional stimulus at their points of origin. Rous *et al.*[17, 18] have discussed this aspect of tumor formation, as encountered in tumors in rabbits produced by tar. Cramer

---

FIGURE 17 (*see opposite page*). To show the response of follicles, deprived of their papilla cells during a quiescent period, to the periodic new follicle growth stimulus. From a mouse which had received thirty applications of 0.3 per cent benzyprene solution during eighteen weeks. (82 dia.)

FIGURE 18. From a mouse which had received thirty-six applications of 0.3 per cent benzpyrene solution during eighteen weeks. No recovery period. Active papilloma formation in progress, with cysts, one of which communicates with the surface. Note adjacent hair follicles in active growth. (28 dia.)

FIGURE 19. From a mouse which had received thirty applications of 0.3 per cent solution of benzpyrene during sixteen weeks, followed by a recovery period of eighteen days. Note contribution to papilloma architecture made by surface communicating cysts, and inactive aspect of the papilloma epithelium. The hair follicles of adjacent skin are inactive. (17 dia.)

and Stowell[22] also made important comments relating to this feature of carcinogenesis induced by methylcholanthrene in mice.

The explanation of focal origin of papillomas awaits better understanding of the conditions which result in destruction of papilla cells. Chance must play a part in the timing of applications to stages of hair growth. The vulnerable stages are those at which the solution has access to the region of the papilla cells, and, therefore, when the hair canal has been formed in active follicles, or after depilation of quiescent hairs. During the development of follicles, at any time before hair canal formation, the papilla cells are not accessible to contact by the solutions.

A discussion of the nature of processes leading to tumor formation is not pertinent to purposes of this paper. My studies in this direction have resulted in agreement with Guldberg's[16] statement that, "In no case have I seen cancer develop without a papillomatous preliminary stage." This conclusion is equivalent to assigning the source of the carcinomas to cells of hair follicle origin. Lacassagne and Latarjet[23] failed to get tumor formation by means of methylcholanthrene applications to hairless scars in the skin of mice. Mider and Morton[24, 25] and Cramer and Stowell[26] have reported the development of carcinomas in mice after single applications of methylcholanthrene to the skin. The strengths of the solutions (0.5 per cent and 0.6 per cent, respectively) and the amounts of solution applied were such as to deliver a relatively large amount of the chemical to the skin. Cramer and Stowell[26] estimated that about 3 mg. of the agent was deposited "to an area of skin extending from the nape of the neck to the middle of the back." Six of fourteen mice which received this amount developed malignant tumors. In an area of the length stated, it is well within the realm of probability that in 6 of 14 mice, there were active hair follicles with open hair canals which permitted the deep penetration of the solution and retention of the chemical. FIGURE 2 of Cramer and Stowell's paper[26] shows cyst formation and remains of a cyst that could only have arisen from active hair follicles with open hair canals. The facts that one mouse developed a "spindle cell" sarcoma and another a "spindle cell" sarcoma and carcinoma can be interpreted only as evidence that the agents reached the depths of the skin *via* the hair canals.

Suntzeff, Carruthers, and Cowdry[27] repeated the single application of methylcholanthrene experiment upon new-born mice (2 to 10 hours after birth) and failed to get tumor formation. They related their lack of success to the undeveloped state of hair follicles and sebaceous glands.

One more bit of collateral evidence of the role of hair follicles in carcinogenesis is the record of failure by Twort and Twort[28] to produce tumors on the soles of the feet in any of 100 mice, which ran daily over a porcelain plate lubricated with shale oil, although 19 did develop tumors elsewhere on different parts of the body.

Summary and Conclusions

As a result of my own studies and a critical review of the literature, the following conclusions seem pertinent for guidance in the interpretation of what has been published and for guidance in planning new studies.

(1) The periodic hair follicle cycle persists in spite of repeated applications of benzpyrene and methylcholanthrene over long periods of time.

(2) The rate of proliferation and growth of the epithelial cells concerned in follicle formation far exceeds these activities in the epidermis of the mouse.

(3) The papilla persists in successive cycles of follicle formation in the sense that, at the end of growth of a follicle, there arises in the papilla a new formation of cells, which accompanies the ascent of the hair and subsequently forms the papilla for the next generation.

(4) Because of the wave-like pattern, in order of initiation of new follicle formation which travels over the skin of the mouse, it has been impossible to know the stage of hair formation at the time of the first, and any succeeding, application.

(5) The result of an application of a carcinogenic hydrocarbon varies greatly in different stages of follicle formation. Applications made after the hair canal has been formed penetrate deeply into the skin and can result in the production of changes very unlike those when the first few applications are made to follicles in the quiescent stage.

(6) Many, if not all, of the changes which have been interpreted as specifically precancerous disappear in the course of weeks after cessation of applications.

(7) Increase in the numbers of hair follicles, that is, new formation from the epidermis, does not take place in the mouse.

(8) Loss of papilla cells is followed by failure of normal follicle formation. The hair matrix does not differentiate. The upper segment of the follicle persists and responds to the periodic stimulus by growth and sebaceous gland formation.

(9) Papillomas take origin from hair follicles that have lost their papilla cells.

(10) Cancers, under the conditions of the experiments I have conducted, have arisen only in papillomas.

Bibliography

1. Stöhr, P. 1903. Entwickelungsgeschichte des menschlichen wollhaares. Anat. Hefte. Abt. i, **23:** 3.
2. Gibbs, H. F. 1941. A study of the post-natal development of the skin and hair of the mouse. Anat. Record **80:** 61.
3. Oyama, R. 1903. Entwickelungsgeschichte des deckhaares der Weissen Maus. (*Mus musculus, varietas Alba*). Anat. Hefte Abt. 1, **23:** 587.
4. Dry, F. W. 1925–26. The coat of the Mouse (*Mus musculus*). J. Genetics **16:** 287.
5. Butcher, E. O. 1934–35. The hair cycles in the albino rat. Anat. Record **61:** 5.
6. Emmens, C. W. 1942. The endocrine system and hair growth in the rat. J. Endocrinology **3:** 64.
7. Unna, K., G. V. Richards, & W. L. Sampson. 1941. Studies on nutritional achromotrichia in rats. J. Nutrition **22:** 553.
8. Cunha, T. J., S. Kirkwood, P. H. Phillips, & G. Bohstedt. 1943. Effect of inositol upon rat alopecia. Proc. Soc. Exp. Biol. and Med. **54:** 236.
9. Haddow, A., L. A. Elson, E. M. F. Roe, K. M. Rudall, & G. M. Timmis. 1945. Artificial production of coat color in the albino rat. Nature **155:** 379.
10. Haddow, A. & K. M. Rudall. 1945. Artificial coat color and the growth of hair. Endeavor **4** (16) (October).

11. Wolbach, S. B. & C. Frothingham. 1923. The influenza epidemic at Camp Devens in 1918. A study of the pathology of the fatal cases. Arch. Int. Med. **32:** 571.
12. Segall, A. 1918. Ueber die entwicklung und den Wechsel der haare beim meer schweinchen (*Cavia cobaya*. Schreb). Arch. f. Micro. Anat. **91:** 218.
13. Glücksmann, A. 1945. The histogenesis of benzpyrene-induced epidermal tumors in the mouse. Cancer Research **5:** 385.
14. Pullinger, B. D. 1940. The first effects on mouse skin of some polycyclic hydrocarbons. J. Path. and Bact. **50:** 463.
15. Cowdry, E. V. 1943. Experimental epidermal methylcholanthrene carcinogenesis in mice. Biol. Symposia **10:** 131.
16. Guldberg, G. 1931. Experimental researches on precancerous changes in the skin and skin cancer. Acta Path. et Microbiol. Scandinavica Suppl. **8:** 1.
17. Rous, P. & J. G. Kidd. 1941. Conditional neoplasms and subthreshold neoplastic states. A study of the tar tumors of rabbits. J. Exp. Med. **73:** 365.
18. MacKenzie, I. & P. Rous. 1941. The experimental disclosure of latent neoplastic changes in tarred skin. J. Exp. Med. **73:** 151.
19. Friedewald, W. F. & P. Rous. 1944. The initiating and promoting elements in tumor production. An analysis of the effects of tar, benzpyrene and methylcholanthrene on rabbit skin. J. Exp. Med. **80:** 101.
20. Friedewald, W. F. & P. Rous. 1944. The determining influence of tar, benzpyrene and methylcholanthrene on the character of the benign tumors induced therewith in rabbit skin. J. Exp. Med. **80:** 127.
21. Silberberg, M. & R. Silberberg. 1947. Hair growths in the skin of guinea pigs painted with 20-methylcholanthrene. Arch. Path. **44:** 297.
22. Cramer, W. & R. E. Stowell. 1943. On the quantitative evaluation of experimental skin carcinogenesis by methylcholanthrene. The factors of dosage time, spacing of applications, and the multiplicity of the carcinogenic response. Cancer Res. **3:** 668.
23. Lacassagne, A. & R. Latarjet. 1946. Action of methylcholanthrene on certain scars of the skin in mice. Cancer Res. **6:** 183.
24. Mider, G. B. & J. J. Morton. 1939. Skin tumors following a single application of methylcholanthrene in C 57 brown mice. Am. J. Path. **15:** 299.
25. Mider, G. B. & J. J. Morton. 1940. Relative importance of local and constitutional effects of methylcholanthrene in production of skin tumors in the mouse. J. Nat. Cancer Instit. **1:** 41.
26. Cramer, W. & R. E. Stowell. 1943. Skin carcinogenesis by a single application of 20-methylcholanthrene. Cancer Res. **3:** 36.
27. Suntzeff, V., C. Carruthers, & E. V. Cowdry. 1947. The role of sebaceous glands and hair follicles in epidermal carcinogenesis. Cancer Res. **7:** 439.
28. Twort, J. M. & C. C. Twort. 1936. The variable sensitivity of different sites of the skin of mice to carcinogenic agents. J. Path. and Bact. **42:** 303.

MEASUREMENT OF THE RATE OF PROLIFERATION OF EPIDERMIS AND ASSOCIATED STRUCTURES*

By W. F. Storey and C. P. Leblond
Department of Anatomy, McGill University, Montreal, Canada

It is widely known that the epidermis undergoes growth and replacement throughout life. Flemming, in 1884, discovered the presence of mitoses in the Malpighian layers of the human epidermis.[1] He realized that the new cells produced by these mitoses exert a pressure which results in a movement of the cells to the region of least resistance, that is, toward the surface. It was soon found that epidermal derivatives, such as the hair and nail, also include a Malpighian layer where mitosis takes place, resulting in an outward displacement of the cells. In both epidermis and its derivatives, the Malpighian cells, on their way out, are transformed into a horny material forming the keratinized layers. This transformation may, however, occur in two different ways:[2] soft keratinization, as in the epidermis itself, in which the cornified cells are spontaneously desquamated and shed from the surface; or hard keratinization, as in nail and hair cortex, in which the cornified material is particularly hard and lengthens indefinitely.

While the evolution of the cells of the epidermis and its derivatives has been known for a long time, much remains to be learned about the rate at which this phenomenon proceeds. The measurements of the rate of proliferation, easily obtained in "hard keratin" structures, are rather difficult in "soft keratin." After briefly discussing the former, most of this report will critically discuss the measurement of the rate of proliferation in cases of soft keratinization.

Hard Keratinization. Since "hard keratin" structures grow indefinitely, their rate of growth can be measured by estimating their increase in size over a definite period of time. Thus, direct measurement is possible in the case of the nail, where growth is continuous.[3] Giroud and Bulliard[4] estimated the rate of growth of human nails. The maximum rate is observed from the age of 5 to 30. The growth rates vary in different locations. Thus, the nails of the fingers grow faster than those of the feet, approximately 1 mm. per week in the former, compared with 0.25 mm. per week in the latter. Also, the different growth rates are related to the size of matrix. Where the matrix is broad and long, as in the thumb, the nail grows 1.20 mm. per week, compared with 0.90 mm. per week in the finger nails, where the matrix is short.

Direct measurement is also possible with the hair: thus, the hairs of the leg grow at the rate of about one and one half mm. a week,[5] and those of the pubic and axillary region grow about 2.2 mm. a week after puberty.[5, 6] Hair growth is continuous only in a few cases, however, such as human scalp and sheep wool. Usually, hair growth is cyclic, as it proceeds for some time until the hair falls and a new hair starts growing again.[5, 7] Thus, Butcher

---

* This work was supported by a grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

showed that, in the rat, a given follicle grows for 17 days and then enters a quiescent period for another 17 days.[7]

Soft Keratinization. In contrast to hair and nail, the epidermis does not increase in size, since the cornified cells are shed as they reach the surface, and the rate of growth cannot be estimated by direct measurement of size or thickness. In fact, the constancy of the thickness of the epidermis in a given area of the body demonstrates that the cell loss by desquamation exactly balances the neoformation of cells by mitosis in the malpighian layers. Presumably, the number of cells of the epidermis remains constant; or, in other words, the epidermis is in a "steady state," regarding its cellular content.

A system in "steady state" may be characterized by the rate of either entry or exit of material, that is, by the amount of material formed or ejected per unit of time. In practice, this rate is often expressed as the "renewal time," that is, the time taken for the replacement of an amount of material equal to the amount present in the whole system. Thus, the renewal time of the epidermis is the time taken for a cell to pass from the lower layers, where it is produced, to the surface, where it is shed.

Before the renewal time of the tissue can be calculated by this means, it is necessary to establish that the assumption of a steady state of the cellular content is correct for any instant of time. W. S. Bullough[8] found, however, that there are diurnal variations in the mitotic activity of the epidermis. Thus, studies of dorsal and ear epidermis of the adult male mouse revealed a double diurnal cycle, with maximal number of mitoses during the periods of rest (*e.g.*, 6:00 A.M. and 2:00 P.M.). Thus, there is no true continuous steady state in this case. It may be noted that in the male, however, there are no variations from day to day.[8–11] The constancy of the epidermal thickness still requires that the total number of cells shed per day be equal to the number of cells dividing per day. There is thus an "average steady state" over the 24-hour period.* An attempt was therefore made to devise a method revealing the number of mitoses taking place over this period of time.

The Colchicine Method. This method may be used to arrest in metaphase the mitoses which take place over a given period of time. Brues[12] presented evidence that colchicine halts all divisions of nuclei at metaphase, without affecting the rate at which these divisions occur. Sentein[13, 14] studied the effect of the drug on the tissues of urodele larvae, anuran larvae, tadpoles, and adults, and in the hypophysis of the guinea pig. He concluded that there is no stimulating effect on mitosis; the drug acts merely to block division at metaphase. Such colchicine-blocked mitoses are incapable of continuing division beyond metaphase as long as the toxic action of the drug persists. After the effect has waned, the nuclei may continue to the completion of their division or, as in most cases, may degenerate. The effect of the drug on various tissues of different animal species was found by Sentein[15, 16] to be consistently of the same nature. Mitotic nuclei are attacked at precisely the same point, and division is blocked at metaphase for the duration of the action of the drug. In another report, Sentein[17] wrote that the effect of colchicine exerts itself at premetaphase, sometimes before the disappearance of the nuclear membrane, but in all cases before the appearance of the spindle, which then does not form. Prophase itself is not impaired.

---

* In the adult female mouse, the mitotic activity of the epidermis varies with the stages of the estrous cycle. The maximum values occur at the end of diestrus and during pro-estrus.[9–11] Presumably, the "average steady state" of females must be considered over the whole estrous cycle.

In work on the intestinal epithelium,[18] it was found that 6 hours was the optimum duration for the action of colchicine, because, up to 6 hours after colchicine injection, there were practically no anaphases and no telophases. Therefore, the mitoses had not progressed beyond the metaphase stage. Also, after the 6 hours, it was found that many of the colchicine metaphases undergo pyknosis and hence could become unrecognizable as such. Bullough[19] reported that, in the mouse, if the tissues were taken at time intervals later than 5 to 6 hours after administration of the drug, there was a decrease in the number of metaphases, which he attributed to an inhibition of mitosis by colchicine after the 6-hour interval. The decrease in the number of mitoses could be only apparent, rather than real, however, due to the decrease in the number of recognizable metaphases, since some mitotic figures might have undergone pyknosis and fragmentation.

The effect of colchicine must begin almost immediately after injection, since it was possible to detect typical colchicine metaphases in the epidermis as early as 30 minutes after injection (unpublished).

In summary, then, it can be assumed that colchicine halts in metaphase all mitoses occurring during the 6 hours between injection and sacrifice of the animals. No stimulation of mitoses occurs during that time interval.

The colchicine method was applied to the plantar epidermis of adult male albino rats weighing about 250 gms. The animals, which were maintained in a room kept at 27° ± 3°C. were injected subcutaneously with 0.1 mg. of colchicine per 100 gms. of body weight, and sacrificed 6 hours later. Counts of resting and metaphase nuclei were made on sections of the plantar epidermis. The number of mitoses was expressed as the percentage of the epidermal cells present as metaphases.

This result supplied the number of mitoses occurring over a 6-hour period. In order to have the total number of mitoses occurring over a 24-hour period, it was necessary to set up four similar groups of animals and inject each one at 6-hour intervals during a day. Thus, the four groups in the experiment reported below were injected at 4:00 P.M., 10:00 P.M., 4:00 A.M., and 10:00 A.M. All groups were sacrificed 6 hours after injection. The sum of the 4 average number of mitoses found in these four groups supplied the average number of mitoses per day.

The Counting of Nuclei. This procedure presents technical difficulties. It is necessary to correct for the errors and obtain a true figure for the number of metaphase and resting nuclei; or, at least, some method of calculating the error must be adopted.

Consider the case where several serial sections of a tissue are made in such a way that the section thickness is less than the average diameter of the nuclei, as in the nuclei of type A in FIGURE 1. When making a count on any one of these sections, the section is viewed in the direction indicated by the arrow. If a count is made of every nucleus and nuclear fragment of type A visible in one section, this count (referred to below as "crude count") will be higher than the true value for the nuclear population of this section, because a nucleus like A can be represented by pieces in as many as three such sections (FIGURE 1, upper left).

If only the center of each nucleus were counted, the result would be the "true" count. The crude count includes, in addition to all nuclei whose centers fall within the section thickness, those whose centers fall within a distance equal to the radius (r) of the average nucleus on each side of the section. It is, therefore, apparent that the ratio of the true count (T) to the crude count (C) will be equal to $\frac{T}{C} = \frac{s}{2r + s}$. Thus, since 2r equals the average diameter (d) of the nucleus, the true count may be obtained by the following formula first devised by Abercrombie:[20]

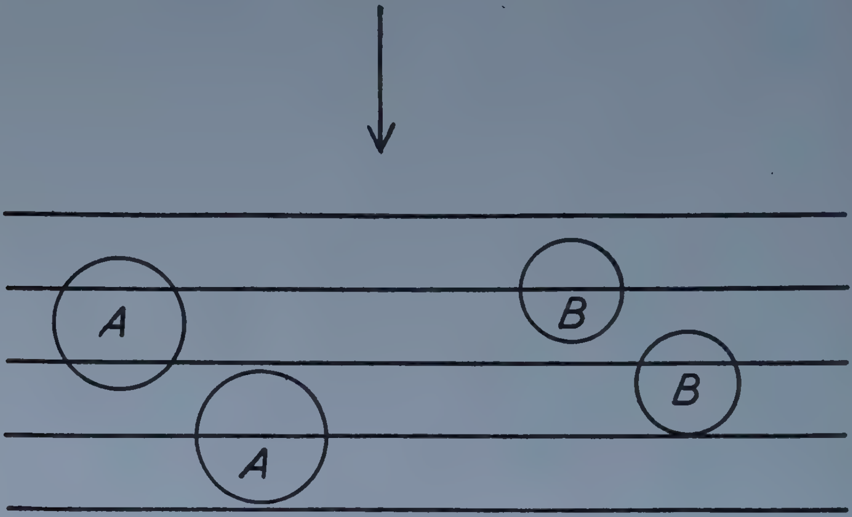


FIGURE 1. Schematic representation of nuclei in a tissue of which several serial sections are indicated by horizontal lines. The arrow indicates the direction in which the sections are viewed through the microscope.

$$T \doteq C \cdot \frac{s}{d + s} \tag{1}$$

where T = true count, C = crude count, d = nuclear diameter, and s = section thickness.

From this formula, it can be reasoned that the figure for the crude count approaches the true value as the section thickness increases, since the value for d remains constant for a given tissue. On the other hand, a decrease in d would decrease the error of the crude count.

Now, the true count of B nuclei, present in the same tissue section as the

A nuclei, can be calculated in the same manner. Thus, if T_1, C_1, and d_1 are the values for the true count, crude count, and diameter of B, respectively, the formula for B is

$$T_1 = C_1 \frac{s}{d_1 + s} \tag{2}$$

and, therefore, the ratio of the number of B to the number of A nuclei is

$$\frac{T_1}{T} = \frac{C_1}{C} \cdot \frac{d + s}{d_1 + s}. \tag{3}$$

It may now be assumed that B corresponds to metaphase nuclei and A to resting nuclei. The number of resting nuclei is so much larger than that of dividing nuclei that, for simplicity's sake, it will be made equal to the total number of nuclei. Therefore, $\frac{T_1 \times 100}{T}$ will be the true percentage of mitosis, and $\frac{C_1 \times 100}{C}$ will be the crude percentage of mitosis. It may thus be calculated that the ratio of the crude to the true percentage of mitosis is equal to $\frac{d_1 + s}{d + s}$ and, therefore, the degree and direction of the error can be accurately estimated.

Renewal Time of the Epidermis. This method was applied to the Malpighian layers of the plantar epidermis, including stratum germinativum and granulosum (FIGURE 2). Thus, the results (TABLE 1) apply only to this portion of the epidermis. Formula (3) shows that the greater the section thickness, the smaller will be the difference between crude and true percentages. Here, the sections were cut at 10μ, because, beyond this thickness, the decreased lighting did not allow for accuracy in distinguishing nuclear fragments. Under the present conditions, the true percentage was 15 per cent greater than the crude value (TABLE 1).

These results make it possible to calculate the renewal time. If 5.24 per cent of the cells will undergo mitosis over a 24-hour period, a number of cells equal to 100 per cent will require $\frac{24}{5.24} \times 100 = 458$ hours, or 19.1 days. The renewal time of the Malpighian layers is therefore 19.1 days. Thus, each epidermal cell will spend an average of 19.1 days travelling outwards through these layers.

Since the nuclear counts for the stratum germinativum and granulosum were recorded separately, it was possible to calculate the renewal time of the stratum germinativum: 16.9 days. By difference from the figure for all Malpighian layers, the renewal time of the stratum granulosum was found to be 2.2 days.

Influence of Temperature. Several series of animals were studied and large variations were obtained for the mitotic activity. The percentage metaphase was found to be higher when the animals were sacrificed during the summer, and lower in the winter. Therefore, an influence of the temperature on mitotic activity was suspected. The plantar skin of the rat is not supplied with an insulating coat of hair, as is the general body surface; hence the tissue would be more influenced by changes in the environmental temperature. The metal wiring of the cage floor must be at a temperature

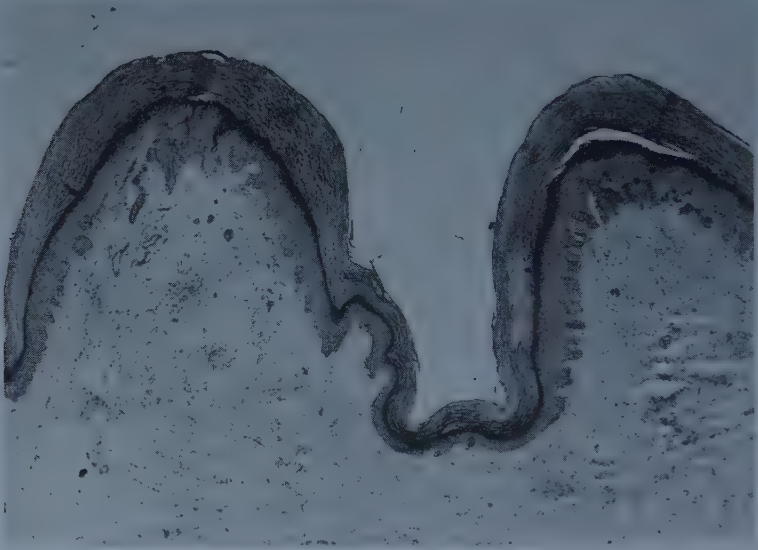


FIGURE 2. Section of foot skin from adult rat cut perpendicular to the surface of the sole of the foot. Note the thickened epidermis over the two plantar pads or thickenings of the skin. The stratum germinativum extends from the epidermis-dermis boundary to the darkly-staining stratum granulosum, above which keratinization begins. The cornified layers extend from the outer limit of the granulosum to the surface. Mitoses occur in the lower third of the stratum germinativum; cell loss takes place from the surface.

TABLE 1

PERCENTAGE METAPHASE IN THE MALPIGHIAN LAYERS OF THE PLANTAR EPIDERMIS OF COLCHICINE-TREATED RATS SACRIFICED AT 6-HOUR INTERVALS THROUGHOUT THE DAY

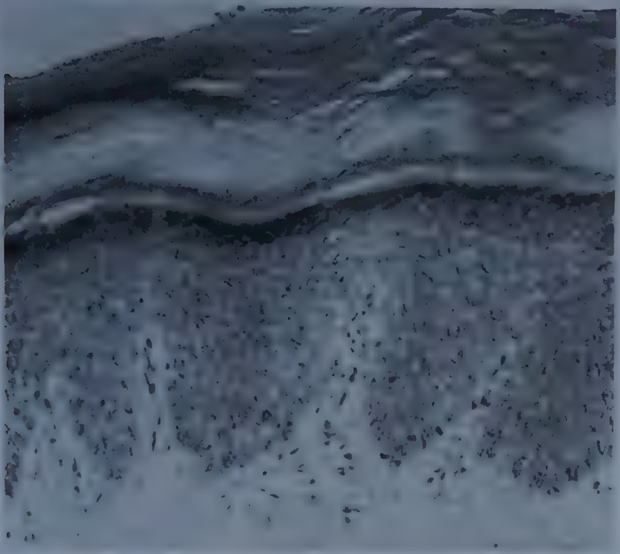
	10:00 A.M. to 4:00 P.M.	*4:00 P.M. to 10:00 P.M.*	*10:00 P.M. to 4:00 A.M.*	*4:00 A.M. to 10:00 A.M.*	*Percentage of the cells dividing over a 24-hour period*
Crude percentage (averages)	1.23	1.07	0.80	1.43	4.53
True percentage (averages)	1.48	1.22	0.91	1.63	5.24

TABLE 2

EFFECT OF TEMPERATURE ON THE PERCENTAGE METAPHASE IN THE MALPIGHIAN LAYERS OF THE PLANTAR EPIDERMIS OF COLCHICINE-TREATED RATS

	10–12°C	*20–22°C*	*25–30°C*
Crude percentage (average)	0.29	0.41	2.65
True percentage (average)	0.33	0.47	2.96

similar to that of the room, and the rat foot, continually in contact with the cage floor, may therefore be affected by changes in the room temperature.



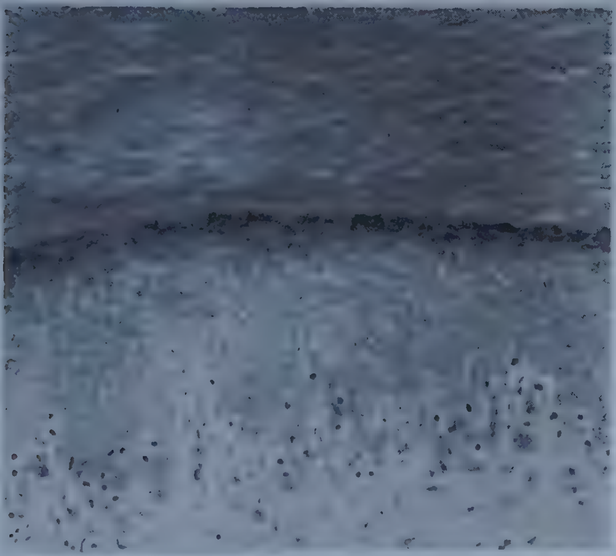


FIGURE 3. (Top.) High power view of the plantar skin kept at 20–22°C for the 6 hours of colchicine treatment (series 2). Three colchicine metaphases are visible in the lower region of the stratum germinativum of the epidermis.

FIGURE 4. (Bottom.) High power view of the plantar skin kept at 25–30°C for the duration of the colchicine treatment. A large number of metaphases are visible in the lower region of the stratum germinativum.

To test this possibility, a method was devised in which the temperature of the cage floor could be read directly for 1 hour prior to, and for the 6-hour duration of, the colchicine treatment.

Fifteen adult male rats were divided into three groups: in the first, the cage floor was kept at 10–12°C; in the second, at 20–22°C; and in the third, at 25–30°C. The animals were placed in their respective cages at 9:00 A.M. and, one hour later, when they were apparently quiet, they were injected with colchicine. Six hours after that, they were sacrificed and the plantar skin taken for histological preparation. Taking the animals at 6:00 P.M. insured that the mitotic activity was roughly comparable to the average for the 24-hour period.[8] Crude nuclear counts were made on one section from each animal as before, and the crude and true percentage metaphase calculated.

The results (TABLE 2) revealed a marked influence of the temperature on mitotic activity. There was only a slight increase in the average values of the 20–22°C group (FIGURE 3) over those of the 10–12°C group. When the temperature was elevated to 25–30°C, however, there was a considerable increase in the mitotic activity of the tissue (FIGURE 4).

When the renewal time was calculated on the basis of the true percentage metaphase, 2.96, for the high temperature groups, it was found to be 8.4 days. With the figure for the percentage metaphase in the stratum germinativum, the renewal time of these layers was calculated to be 7.3 days; and in contrast, that of the stratum granulosum, 1.1 days. This means that, when the environmental temperature is elevated, the renewal time of the epidermis is much decreased. But these results imply that the tissue thickness remains constant (an assumption which remains to be verified in this special case). If it is so, and if the rate at which cells are supplied is increased, then the rate of cell loss must also be increased.

Conclusions

The rate of growth of hard keratin structures, such as hair, cortex, and nails, may be measured by estimating their increase in length. In the case of soft keratin structures, such as the epidermis itself, there is no increase in size, since the cornified cells desquamate as they reach the surface. There is a balance between cell production and cell loss, resulting in a constant thickness of the epidermis for a given area. In other words, the cellular content of the epidermis is in a "steady state."

The rate of proliferation of structures in "steady state" may be expressed by the time taken for their replacement or "renewal time." This was estimated for the Malpighian layers of the plantar epidermis and found to be 19.1 days. Of this time, 16.9 days are taken for the renewal of the stratum germinativum, and 2.2 days for that of the granulosum.

While these results were obtained in a room kept at a temperature of 27° ± 3°C, an experiment with accurate control of the temperature of the cage floor showed that the rate of renewal of the epidermis is dependent on the external temperature. The renewal of the epidermis is less rapid at low temperatures and more rapid at high temperatures.

Bibliography

1. Flemming. 1884. Quoted by Branca. Ref. 3, pp. 102–103.
2. Leblond, C. P. 1950. Histological structure of hair, with a brief comparison to other epidermal appendages and epidermis itself. Ann. N. Y. Acad. Sci. **53** (3): 464.

3. Branca, A. 1912. Le tégument externe et ses dérivés. Traité d'Anat. Humaine. **5:** 102–113. Masson et Cie. Paris.
4. Giroud, A. & H. Bulliard. 1930. La kératinisation de l'épiderme et des phanères. Genèse des substances soufrées de la kératine. Arch. de Morph. gén. et exp. Gaston Doin et Cie. Paris.
5. Trotter, M. 1932. The hair. Special Cytology, by E. V. Cowdry. **1:** 41–65. Paul B. Hoeber. New York.
6. Hamilton, J. B. 1949. The relationship of the growth of axillary hair to age, sex, gonadal secretions and titers of urinary steroids. Anat. Rec. **103:** (Suppl.): 47.
7. Butcher, E. O. 1934. The hair cycles in the albino rat. Anat. Rec. **61:** 5.
8. Bullough, W. S. 1948a. Mitotic activity in the adult male mouse, *Mus musculus* L. The diurnal cycles and their relation to waking and sleeping. Proc. Roy. Soc. Ser. B. **135:** 212.
9. Bullough, H. F. 1943. Cyclic changes in the skin of the mouse during the estrous cycle. J. Endocrinol. **3:** 280.
10. Bullough, W. S. 1946. Mitotic activity in the adult female mouse, *Mus musculus* L. A study of its relation to the estrous cycle in normal and abnormal conditions. Phil. Tr. Roy. Soc. London, Ser. B., Biol. Sc. **231:** 453.
11. Bullough, W. S. 1950a. Epidermal mitotic activity in the adult female mouse. J. Endocrinol. **6:** 340.
12. Brues, A. M. 1936. The effect of colchicine on regenerating liver. Proc. Physiol. Soc. **86:** 1.
13. Sentein, P. 1942a. Action de la colchicine sur la division cellulaire au cours du développement du Triton. Montpellier méd. **21–22:** 491.
14. Sentein, P. 1942b. Mise en evidence de mitose dans l'hypophyse du cobaye par l'action de la colchicine. Variation de l'activité divisionelle à l'état normal et après injection d'hormone gonadotrope. Montpellier méd. **21–22:** 493.
15. Sentein, P. 1943a. Action de la colchicine sur les mitoses de maturation chez le Triton. Compt. rend. Soc. de biol. **137:** 132.
16. Sentein, P. 1943b. Mode d'action de la colchicine sur la caryocinése. Compt. rend. Soc. de biol. **137:** 133.
17. Sentein, P. 1946. L'association de la colchicine à la radiothérapie dans la thérapeutique des tumeurs malignes. J. de Radiol. et d'Elect. **27:** 540.
18. Leblond, C. P. & C. E. Stevens. 1948. The constant renewal of the intestinal epithelium in the albino rat. Anat. Rec. **100:** 357.
19. Bullough, W. S. 1949. The action of colchicine in arresting epidermal mitosis. J. Exp. Biol. **26:** 287.
20. Abercrombie, M. 1946. Estimation of nuclear populations from microtome sections. Anat. Rec. **94:** 239.

THE DEVELOPMENT OF PELAGE HAIRS AND VIBRISSAE FROM SKIN IN TISSUE CULTURE

By Margaret H. Hardy*

Strangeways Research Laboratory, Cambridge, England, and Tissue Culture Laboratory, University of Texas Medical Branch, Galveston, Texas.

Hair is recognized as one of the most sensitive indicators of bodily health in man and animals; but, although we know that many factors in heredity and environment affect the formation and growth of hair, we do not understand how they act. We need first of all to find out which effects are due to direct action on the skin and which are secondary to other physiological changes. For this reason, great interest centers on experimental methods of modifying the environment of skin.

Skin grafting is a valuable tool in the analysis of hair follicle behavior, and use has been made of autografts,[1, 2] homografts,[3] and even interspecific grafts.[4, 5] It is not yet possible to obtain permanent homografts of adult skin.[6] Two important advantages are obtained by removing the skin to an even more unnatural environment, a tissue culture chamber: first, the complexities of a continually changing blood supply are avoided, and second, the day by day observation of living follicles is made possible.

Two kinds of hairs have received attention from those who attempted to cultivate skin and hair follicles *in vitro*. They are the tactile hairs, or vibrissae, which are found in most mammals on the face and at other specific sites, and the pelage hairs, which cover the general body surface. The vibrissae are larger and more specialized hairs with a sensory function.[7, 8] They are usually the first to develop in the embryo, and this gives them some advantages as material for tissue culture. Although intermediate forms do occur,[9] it is easy in practice to distinguish vibrissae from pelage hairs.

It is well known that the tissues from embryos will differentiate more readily *in vitro* than will those of adults, and, for that reason, the study of hairs in tissue culture began with embryonic skin. Strangeways[10] was the first to do this. She reported in a short note that pelage hair follicles from guinea pig embryos underwent a little development in tissue culture, but that vibrissa follicles gave more satisfactory results. Murray[11] indicated in an abstract that she had cultivated the vibrissa follicles of rat embryos and obtained good differentiation and hair production. Both authors[10, 12] were less successful with the later developing pelage hair follicles, and attributed their failure to the thickening and keratinization of the epidermis, which discouraged further development of the follicles.

Hardy[13] explanted skin from the trunk of mouse embryos, and this tissue underwent all the stages of normal follicle differentiation, which led to the production of keratinized pelage hairs. This development was observed in living explants but was demonstrated only by the comparison of stained sections of normal and cultivated mouse skin.

---

* Permanent address: C.S.I.R.O., McMaster Laboratory, Parramatta Road, Globe, N.S.W., Australia. This work was begun at Cambridge on an Overseas Studentship of the Commonwealth Scientific and Industrial Research Organization, and continued in Galveston under a grant from Hoffmann-La Roche Inc., administered by Dr. C. M. Pomerat.

Some further observations have been made on the follicles of both pelage hairs and vibrissae *in vitro*. It is the purpose of this paper to describe and illustrate some features of the development of both types of follicle as seen in living, unstained explants of mouse embryonic skin, and also to discuss the relevance of tissue culture studies to the problems of hair follicle development.

Material and Methods. For the tissue culture experiments, inbred albino mice were kindly supplied by Professor R. F. Blount of the University of Texas Medical Branch, Galveston, Texas. Explants of skin of the trunk (epidermis and mesoderm) from 13-, 14-, and 15-day mouse embryos were grown in hanging drop cultures. The skin pieces were placed on the surface of a clot made from equal parts of fowl plasma and chicken embryo extract. The technique of removal of the skin has already been described.[13] The orientation of explants is indicated in FIGURE 1. The skin from 13- and 14-day embryos curled up, after a few days of cultivation, in the manner indicated in the diagram, with the dermis surrounding the epidermis. Most explants from the 15-day embryos curled in the opposite direction, with the epidermis outside.

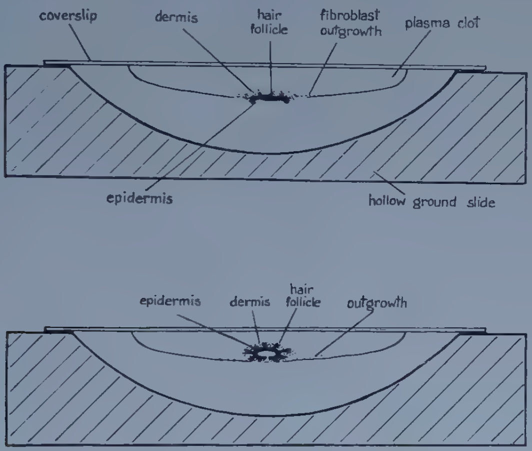


FIGURE 1. The orientation of explants of body skin in hanging drop cultures. Cross-sections through the center of culture chambers are represented. In the upper diagram, the skin is on the surface of the clot and the epidermis is beginning to curl at the edges. The lower diagram represents the same culture several days later, when the epidermis has rolled into a ball.

For the study of vibrissa follicles, pieces of upper lip skin of 14- and 15-day embryos, each containing from 1 to 20 follicle rudiments, were placed on the same medium. They were usually explanted with the long axes of the follicles in the plane of the clot surface, and most follicles continued to grow in that plane. Each explant of skin from the trunk or lip was cut out of its clot and placed on a fresh clot every three or four days. Cultivation was continued for 5 to 14 days.

Photographic records of the living explants were made daily with the aid of a bellows system attached to a Spencer microscope. A 16 mm. Spencer achromatic objective was used for photographs at a magnification of ×150, and an 8 mm. Leitz apochromatic objective for photographs at ×500. The hanging drop culture chamber was placed on the stage and the microscope was focussed through the cover glass and plasma clot to the explant. It was possible to obtain good definition at various levels in the explant, but naturally the best results came from focussing on the surface nearest the microscope. With this apparatus, pictures were obtained of pelage hair follicles and vibrissa follicles viewed laterally and from the base. A few explants placed with the epidermis next to the clot provided photographs of follicles as seen from the epidermal surface.

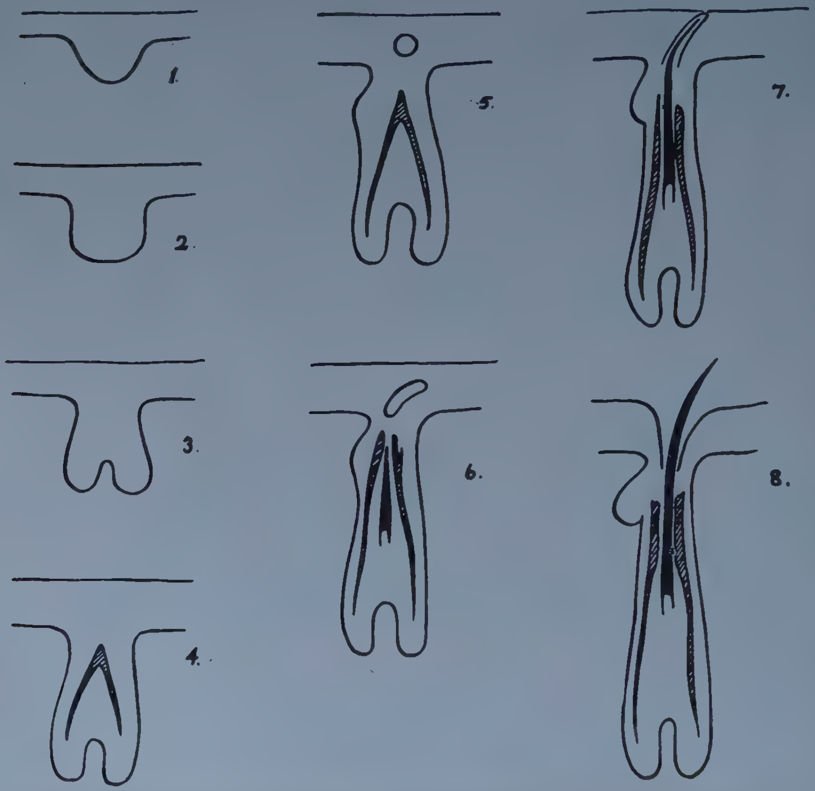


FIGURE 2. Diagram of stages in the development of pelage hair follicles of the mouse, indicating only the most prominent features in the epidermal part of the follicles. The hatched area is the hardened and birefringent portion of the inner root sheath, *viz.*, Henle's layer and the upper part of Huxley's layer. The solid black area is the keratinized portion of the hair. A hair canal is indicated in Stages 5 to 7.

Many of the explants and some embryonic skin pieces for controls were fixed in Zenker's fluid. Some were stained as whole mounts with haematoxylin, and others were sectioned serially at 8μ and stained with haematoxylin, eosin, and picric acid.

The Development of Pelage Hair Follicles in Vitro. The stages in the development of body hair follicles in embryos and young mice have already been described.[13] The main features are summarized in FIGURE 2. On the lateral part of the trunk, Stage 1 of the first follicles occurs at 14 days, Stage 2 at 15 days, Stage 3 at 16 days, Stage 4 at 19 days, Stage 5 at 20 days, Stage 6 at 21 days or the time of birth, Stage 7 at one day after birth, and Stage 8 at two to three days after birth. Thus, the whole sequence occupies about ten days. The layers of the epidermis begin to differentiate from the ectoderm at about 15 days, and the stratum corneum first appears at 18 days. The parts of the epidermis and of a fully differentiated and active hair follicle are indicated in FIGURE 3.

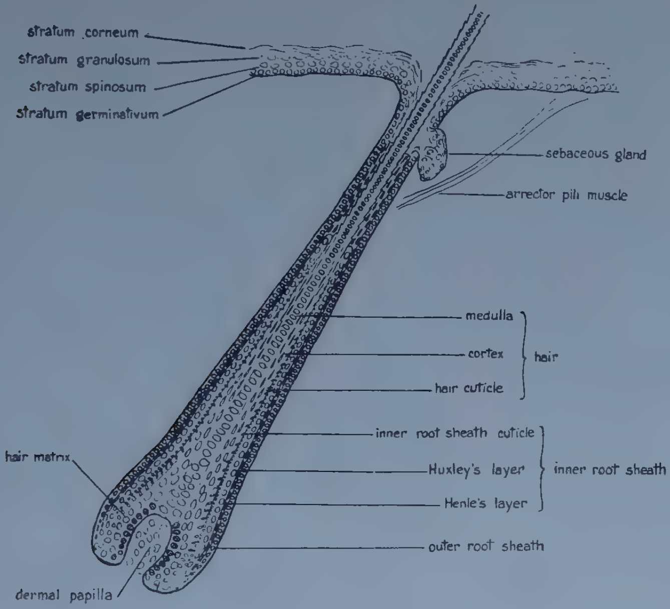


FIGURE 3. Diagram of a fully developed and active pelage hair follicle in the mouse.

In the earlier paper,[13] it was shown that follicle differentiation took place in about 70 per cent of explants of skin from 12- to 14-day embryos, and Stage 8 was reached in many of these. The histological details, and, in many cases, even the rate of development and the size of the follicles, were normal. The present observations are purely qualitative, however, and their aim is to record the sequence of events rather than the normal rate of progress. Many of the explants illustrated were differentiating a little more slowly than embryonic tissue. This may have been due to minor differences in technique, or to the long periods of exposure of the explants to room temperature for microscopic examination and photography. Although development was slow, it took a normal course.

The differentiation of the epidermis and the formation of hair follicles in normal skin are always preceded by an event which will be described as organization at the basement membrane which separates the dermis and epidermis. This feature is seen very clearly in living explants, where the basement membrane appears first as a sharp line separating the central compact translucent epidermis from the darker and more scattered dermal elements (PLATE 1, (a)). Frequently, the epidermal cells touching the basement membrane are oriented with their long axes perpendicular to the membrane. Prior to this organization, which takes place at 13 or 14 days in normal development, the ectoderm appears as a layer resting on the mesoderm, not separated from it by a distinct membrane, and not differing markedly from it in light absorption.

The pattern of hair follicles in the skin of mammals has been discussed in an earlier paper in this monograph.[14] The first pelage follicles that appear in the mouse embryo are regularly spaced and arranged more or less in rows. After a few days, new follicles form in the spaces between them. The same pattern was seen in sections of skin explants,[13] but it is best illustrated from the living tissues. The formation of the first follicles in the epidermis is illustrated in PLATE 1, (a) and (b), while (c) and (d) show in another explant the formation of new follicles between the old ones. It will be noted that the spacing of follicles is very regular and is similar in the two explants. In the mouse, each of these follicles may become the center of a group of younger follicles which begin to appear four days after birth; but the formation of such groups has not been observed in tissue culture.

PLATE 1 also illustrates the synchronous development of the follicles within an explant. In (b) all the follicles are at Stage 1 or 2. In (c) the follicles are shown at Stage 2, and in (d) the same follicles a day later are at Stage 3 and it is possible to distinguish some of the dermal papillae as small circles within the larger circles of the epidermal plugs. The concentric arrangement of dermal tissue around the base of the follicles is seen in (b) to (d).

A more detailed picture of the development of individual follicles is obtained by examining the living explants at higher power. The follicle depicted on successive days in PLATE 2, (e) and (f), shows clearly the outline of a dermal papilla made up of polygonal cells. These tightly packed cells differ in appearance from the fibroblastic cells in the rest of the dermis. The basement membrane of the epidermis is continued around the epidermal part of the follicle and covers the dermal papilla. Movements of the papilla cells, or, more probably, of the rapidly dividing epidermal cells of the hair matrix, may account for the frequently irregular outline of the membrane in this region ((e) and (f)). The increase in length of the dermal papilla in (f), as compared with (e), is part of the gradual elongation which takes place during the differentiation and activity of the follicle.

The tendency noted above for epidermal basal layer (stratum germinativum) cells to become oriented with respect to the basement membrane was observed also in the hair follicles. In PLATE 2, (e), the follicle is at Stage 4, and the cells destined to become the outer root sheath have their long axes at right angles to the basement membrane. Cells of the hair matrix surrounding the dermal papilla show similar orientation. Inside the follicle

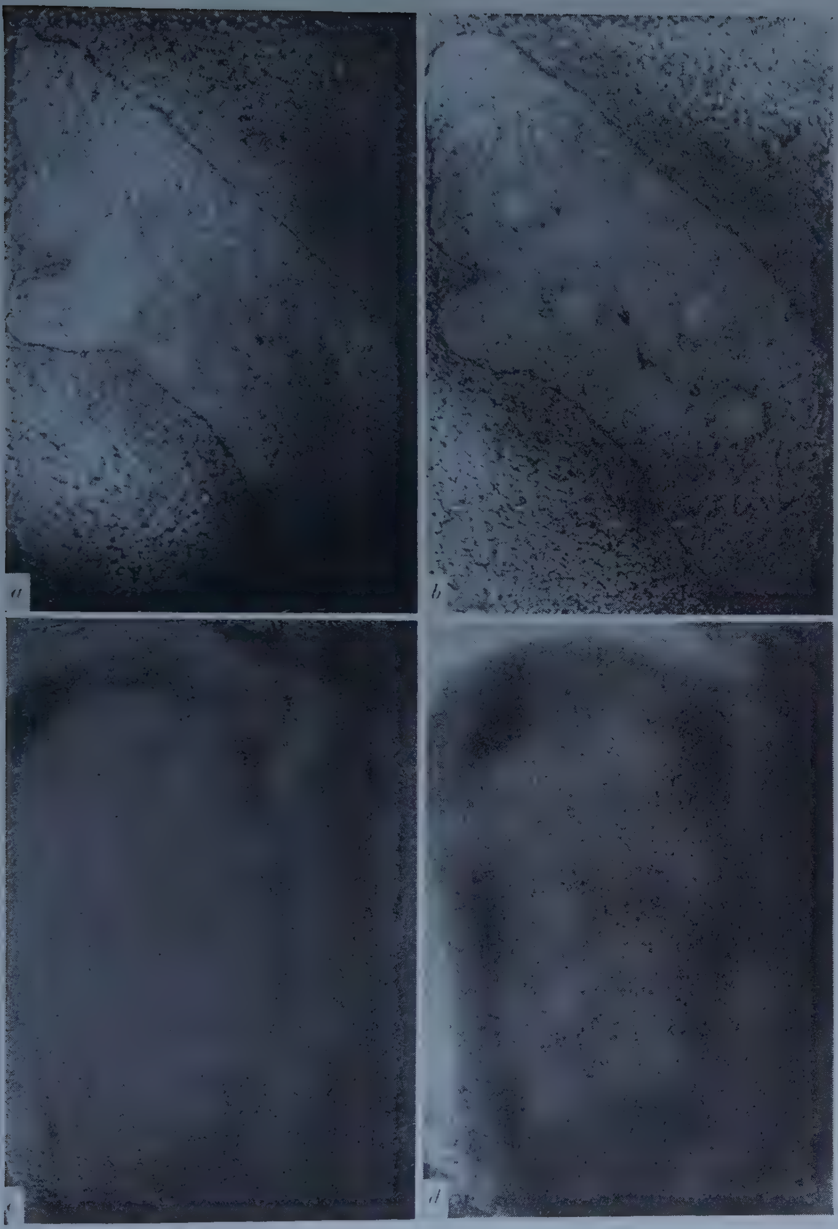


PLATE 1.

(a) Explant of body skin from 14-day mouse embryo after two days *in vitro*. No follicles have appeared. ×106.

(b) The explant shown in (a) two days later, *i.e.*, after four days *in vitro*. Five regularly spaced follicles have appeared. Note also the increase in area of the epidermis and the rounding of its outline. ×106.

(c) Explant of skin from 15-day embryo after three days *in vitro*, with evenly spaced, early developing follicles. (From left to right, No. 1 in the upper row, Nos. 2–3 in the second row, Nos. 4–6 in the third row.) The dark lines in the background are due to the thickening and curling of the epidermis. ×106.

(d) The explant shown in (c) one day later, *i.e.*, after four days *in vitro*, showing new follicles forming between the early ones (*e.g.*, in the triangle between follicles 2, 4, and 5, and in the triangle between follicles 2, 5, and 6). ×106.

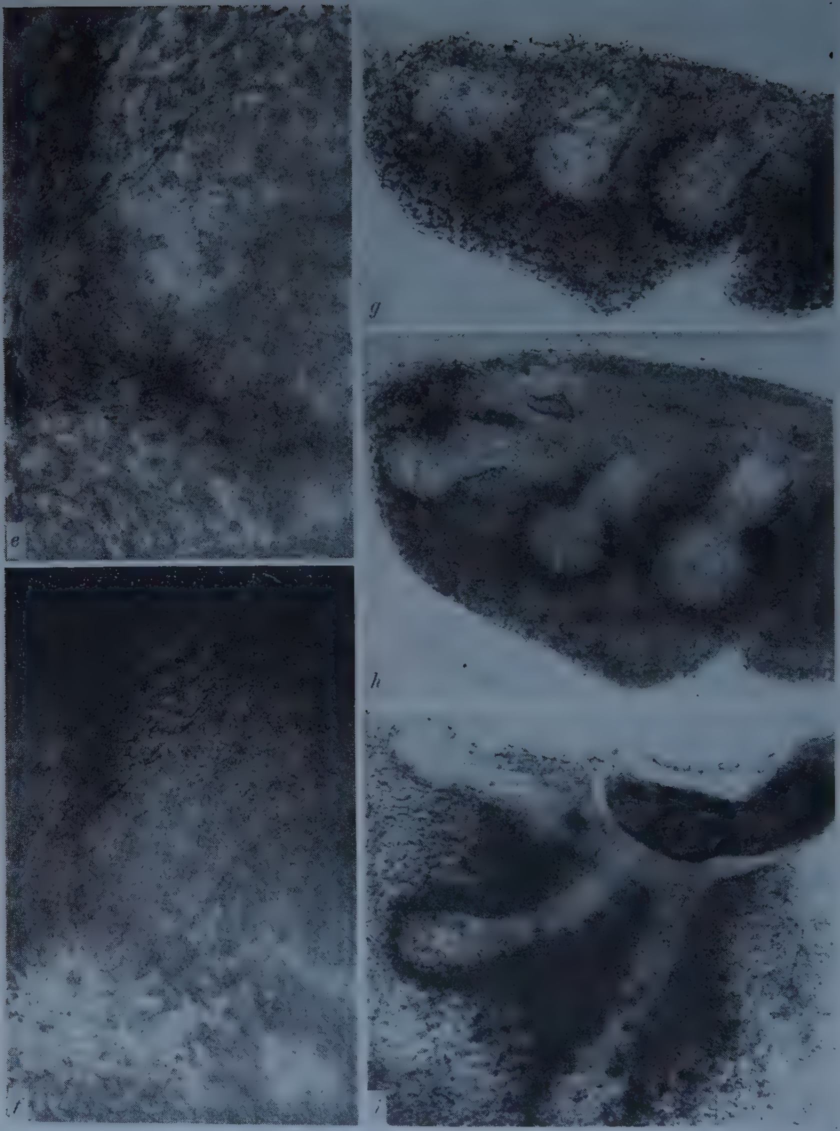


PLATE 2.

(e) Pelage hair follicle in explant of skin from 13-day embryo after six days *in vitro*, now at Stage 4. ×355.

(f) The follicle shown in (e) one day later, *i.e.*, after seven days *in vitro*, at about Stage 5. ×355.

(g) Explant of skin of upper lip from 14-day embryo after one day *in vitro*, showing three vibrissa follicles at Stages 1–3. ×106.

(h) The explant shown in (g) after three days *in vitro*, with follicles at Stages 3-4. The dermal papilla can just be distinguished in the central follicle. ×106.

(i) The explant shown in (g) after seven days *in vitro*, showing long follicles, two at Stage 7. The follicle on the right shows Henle's layer as a clear line on either side of the follicle, with the keratinized hair as a clear double line in the center. ×106.

in (e), there is a short cone of elongated cells, the beginning of the hair cone of inner root sheath material. The swelling on the left side of the follicle is probably the sebaceous gland anlage. The changes that have taken place one day later are illustrated in (f), in which the follicle is probably at Stage 5. The whole follicle has elongated, as can be seen by observing the distance from the lateral swelling to the dermal papilla; but the hair cone has become even longer, and its tip is near the right hand margin of the photograph. Many of the elongated cells of Henle's layer are clearly seen, and there is a column of more amorphous cells inside the hair cone where further differentiation is taking place to form a hair.

Development of Vibrissa Follicles in Vitro. The histogenesis of facial vibrissa follicles is essentially the same as that of pelage hairs. It is possible from the first, however, to distinguish between the two follicle types in the mouse embryo. Facial vibrissa follicles are the first to develop and are consistently longer and wider than the others. Each vibrissa follicle has a dense dermal sheath from the earliest stage, and, from Stage 3 onwards, the epidermal part of the follicle is hour-glass shaped (FIGURE 4). The follicles of pelage hairs in the mouse do not at any time have a distinct dermal sheath, and the epidermal portion is more cylindrical (FIGURE 2). The vibrissa follicle of the adult mouse has thickenings of the outer root sheath and a complex connective tissue sheath with blood sinuses and special nerve endings. It is similar to that of the rat, described by Vincent.[7]

There has been no detailed study of the time relations in histogenesis of the vibrissa follicles in the mouse. From a few original histological observations, supplemented by descriptions of external features,[15] it seems that the most advanced follicles on the upper lip are at Stage 1 at 12 days, Stage 2 at 13 days, Stage 3 or 4 at 15 days and Stage 8 at 17 days. Thus, a single vibrissa follicle develops from Stage 1 to Stage 8 in five days, while a pelage hair follicle takes nine or ten days for this development. On the upper lip, there are five rows of vibrissae, each with five to seven follicles. There is a ventro-dorsal gradient in the time of development of the rows, and also a latero-mesial gradient in both time of development and size of follicles.[9, 15]

In the present study, the explants of upper lip skin from 14- and 15-day embryos went through all the eight stages of follicle development described above, and keratinized hairs were produced *in vitro* from late-developing follicles, which had not even begun to form at the time of explantation. Those follicles which were already present when cultivation began, also underwent normal growth and differentiation.

PLATE 2, (g), (h), and (i), shows an explant from a 14-day embryo after one, three, and seven days, respectively, *in vitro*. The largest follicle is at Stages 3, 4, and 7, respectively. The characteristic hour-glass shape of the epidermal part of the follicle which is illustrated in FIGURE 4 may be seen also in (h). All three photographs show the dense dermal sheath, and the basement membrane surrounding the epidermal part of the follicle is clearly indicated. In this and other explants, the gradient in the time of development of follicles in a row was maintained throughout the period of cultivation, just as it is *in vivo*. An inner root sheath (Henle's layer) and a hair are sharply defined in the follicle on the right in (i). The differentiation of the epidermis proceeded normally in these explants, as it does in explants of skin from the trunk. The keratinization of the epidermis has just begun in (h), and in (i) there is a large area of stratum corneum.

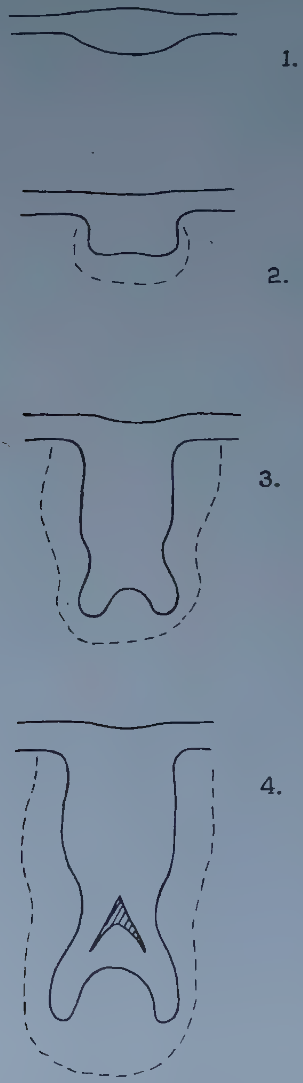


FIGURE 4. Early stages in the development of a vibrissa follicle in the mouse. The hatched area is the hardened and birefringent portion of the inner root sheath. The dotted line indicates the extent of the dermal sheath.

The examination of vibrissa follicles under higher power reveals more structural details, some of which are shown in PLATE 3, (k). This follicle

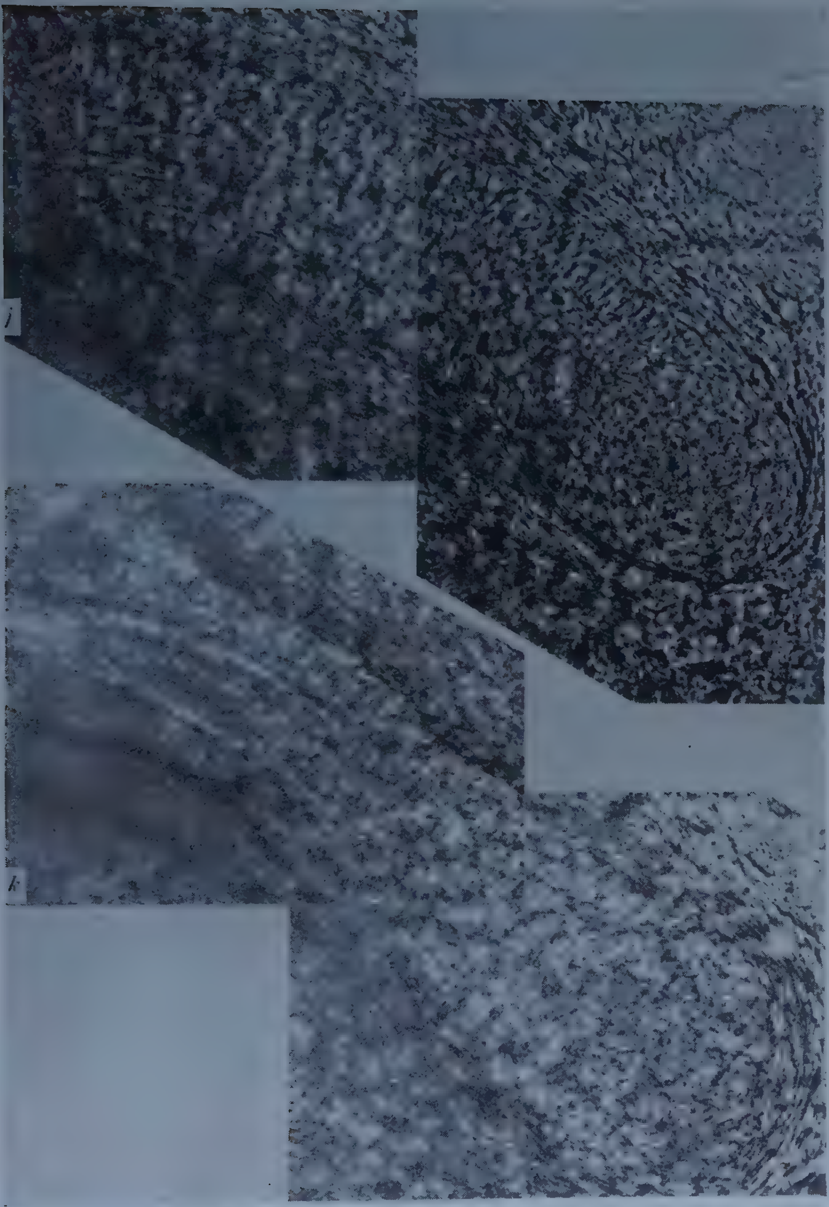


PLATE 3.

(k) Vibrissa follicle in explant from 15-day embryo after seven days *in vitro*, showing complete differentiation. ×355.

(j) The same follicle as in (k), after nine days *in vitro*. ×355.

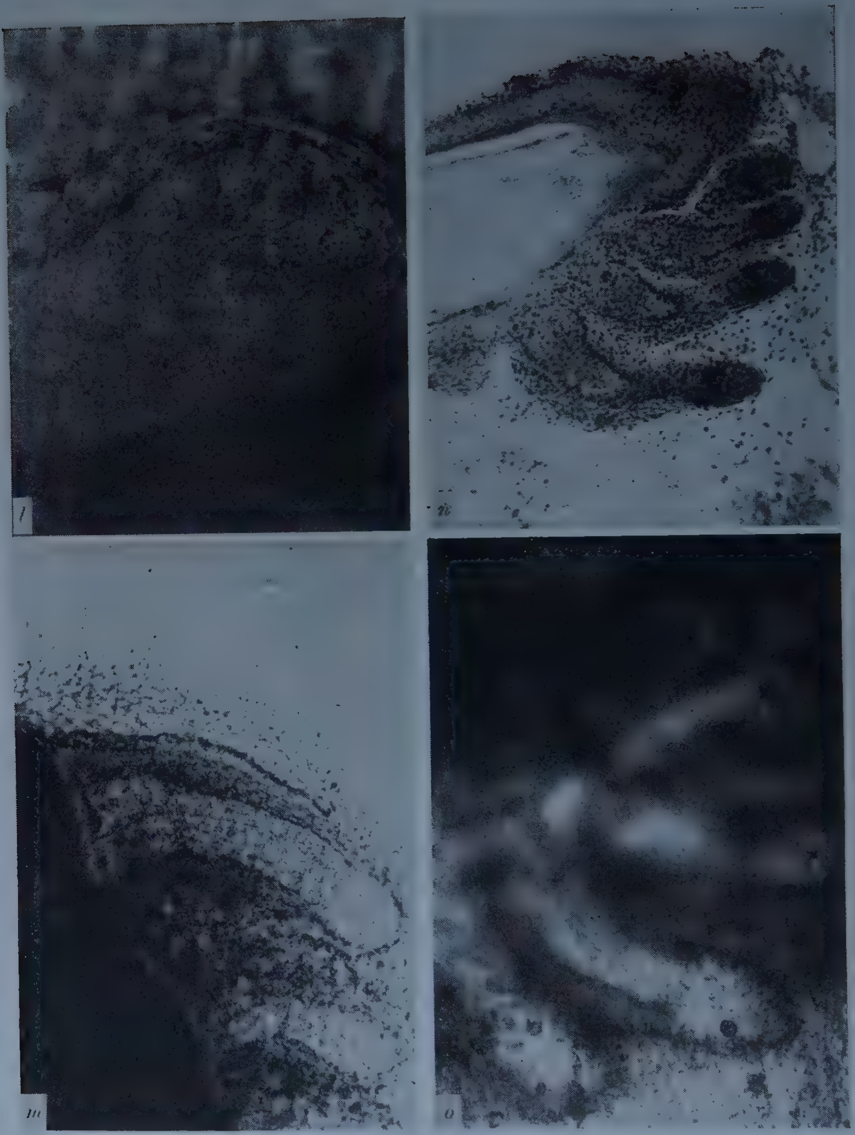


PLATE 4.

(l) The basal portion of the vibrissa follicle shown in PLATE 3 after only five days *in vitro*. ×355.

(m) A low-power view of the follicle shown in PLATE 3 (after nine days *in vitro*) with adjacent follicles. Note the sharply defined Henle's layer in the largest follicle, and the outline of the hair. ×106.

(n) A section 8μ thick, stained with haematoxylin, eosin, and picric acid, from the same explant as in (o), fixed after nine days *in vitro*. Note the outer root sheath with cells oriented at right angles to the basement membrane, and the differentiated sebaceous cells at the follicle necks in the regions marked by gland swellings in (o). ×106.

(o) Explant from upper lip of 14-day embryo after eight days *in vitro*, with vibrissa follicles. Note the sebaceous gland swellings at the necks of the follicles. ×106.

is in an explant from a 15-day embryo after seven days *in vitro*. It is possible to distinguish in it the following layers: a thick connective tissue sheath with many fibres parallel to the long axis of the follicle, a distinct basement membrane, an outer root sheath in which the cells adjacent to the basement membrane are oriented at right angles to it, Henle's layer with elongated cells forming a distinct hyaline layer, indications of Huxley's layer, and a small hair forming in the center. In PLATE 3, (j), which shows the same follicle two days later, all of these structures are seen, but Huxley's layer, with rounded cells, and the outline of the keratinized hair are much more distinct.

Normal hair follicles have dermal papillae of various shapes which are probably related to their functional state. In explants, it is possible to study the changes in papilla form which take place in a single follicle from time to time.

PLATE 4, (l), shows the follicle illustrated in PLATE 3 after only five days *in vitro*. The dermal papilla is rounded, and consists of unoriented cells. There is a distinct basement membrane and the adjacent hair matrix cells are oriented at right angles to it. In PLATE 3, (k), two days later, the papilla is much more conical, the basement membrane is still distinct, and the hair matrix cells are oriented. In PLATE 3, (j), the papilla is more elongated, the basement membrane is less distinct at the sides and not visible at the tip of the cone, and the orientation of hair matrix cells is somewhat disturbed. These three photographs of the papilla were taken, respectively, when the follicle was differentiating, when it was functioning actively, and when it was ceasing hair production. The connective tissue fibres which appear in PLATE 3 around the follicle do not form inside the dermal papilla.

In PLATE 4, (m), the follicle shown in PLATE 3, (j), is seen at a lower power, beside some smaller follicles. The gradient in the time of development of the follicles in a row is again apparent. This figure shows, in the largest follicle, the extent of the dermal sheath, the sebaceous gland swelling towards the upper margin of the photograph, and the hair on the left with a zigzag form that suggests it had difficulty in penetrating the thick keratinized epidermis.

It is instructive to compare the living explants with stained serial sections made from the fixed tissue, and this has been done with many cultures. PLATE 4, (o), shows a living explant from a 14-day embryo after eight days *in vitro*, and (n) a stained section from the same explant, fixed after nine days *in vitro*. In (o), five vibrissa follicles (from left to right, nos. 1 to 5) show the usual gradient in degree of development. Most of follicle no. 1 of (o) is absent from the section in (n), but follicles 2 to 5 are cut more or less longitudinally. Both figures show the basement membrane. The dark mass in the skin in the upper part of (o) is seen in (n) to consist of stratum corneum, and beneath it, in the latter figure, the stratum granulosum, stratum spinosum, and stratum germinativum can be distinguished. The dark hair canals in (o) are seen, from the section in (n) and from others adjacent to it, to be formed by the keratinization of epidermal cells. They are therefore similar to the hair canals of pelage hair follicles which have already been described.[13] The outline of a hair is shown clearly in follicle no. 2 in (o). Only traces of hairs are seen in follicles 2 and 3 in (n), but there is a more nearly longitudinal section of the hair in follicle no. 4. In (n) also, several mitoses may be distinguished in the hair matrix of different follicles.

Discussion

From the tissue culture studies, it appears that the dermis and epidermis together are capable of forming and maintaining hair follicles, and that no other cellular structures are required. We do not know, however, how the dermal and epidermal layers influence one another in the skin or in the hair follicles. Medawar[6] made an interesting observation on the behavior of small skin autografts on a large granulating area in rabbits. He noted that, when epithelial sheets grew out from the donor epidermis over the host dermis, new groups of hair follicles were formed by the proliferation of cells from the epithelial sheets. The arrangement of follicles in groups was similar to that of normal skin, and this suggests that the position, and also the initiation, of hair follicles may be influenced by the dermis. In the present study of mouse embryonic skin, parts of explants from which either the epidermis or the dermis had been accidentally cut away showed no trace of follicle or papilla development. The induction of the epidermal component of a feather follicle by the dermal component has been demonstrated by grafting,[16] and the way is open for a similar investigation of hair follicles.

The organization at the basement membrane, which precedes epidermal differentiation and follicle development, has already been described. In a few explants in the present experiments and earlier ones,[13] this organization did not take place, and, despite the presence of ectoderm and mesoderm, follicles were never formed. Miszurski[17] noted in explants of chicken embryonic skin a preliminary change corresponding to the organization of mouse skin. Perhaps this change is a prerequisite for the differentiation of epidermal structures.

Transplantation studies give some indication of the time at which follicle development is determined in embryonic skin. Thus, Hiraiwa[4] found follicle development took place in grafts of anterior and middle thirds of 11-day rat embryos on the chorioallantoic membrane of the chick. The advanced follicle, in an explant from an 11-day rat embryo figured by Hiraiwa (Figure 5 on p. 450), is almost certainly a vibrissa follicle. Waterman[18] found hair follicles in some grafts from 12- to 16-day mouse embryos to the omentum of the adult rabbit. In grafts of "skin and such underlying tissues as may have been included by accident" from a 15-day mouse embryo on the chick chorioallantoic membrane, Reed and Alley[19] found that follicles were formed. Rawles[5] obtained follicle development in pieces of tissue, including skin, from 8-day mouse embryos grafted to the chick coelom. All these transplants included tissues other than skin, and so did the explants of ventral body wall from 10-day mouse embryos, which also produced follicles after cultivation.[13] It seems probable that both pelage hair and vibrissa follicle formation in rat embryo skin was determined by

11 days, and pelage hair follicle formation in the mouse by eight days. In the experiments cited, however, the possibility of later influences from the adjacent tissues cannot be excluded. Tissue culture experiments have shown that the skin alone from mouse embryos, at least as young as 12 days, is determined for body hair follicle formation,[13] and that upper lip skin is determined for the formation of all the vibrissa follicles from at least the 14th day.

The cause of the initiation of hair follicles in embryonic skin is unknown. From the tissue culture experiments,[10, 11, 13] it may be deduced that neither circulating blood nor nervous stimulation is essential for follicle formation. Since the time of follicle formation in an explant is related to the total age of the tissue, rather than to the duration of cultivation, it seems most likely that any necessary stimulus resides within the skin. An alternative is that an outside stimulus which happens to be present in the culture chamber acts on the skin only after the skin has been made receptive by factors within itself.

The skin of the trunk of a 12-day mouse embryo has the potentiality, not only to form early hair follicles during cultivation, but also to undergo complete differentiation of follicles, with the growth of keratinized hairs. The follicles which formed in explants from 10-day embryos[13] only developed to Stage 3. It is probable, however, that this was not due to lack of potentiality, but to other factors, since Rawles[5] obtained hairs from her coelomic grafts of 8-day mouse tissues. Reed and Alley[19] found one hair in a chorioallantoic graft from a 15-day mouse. There is less information about the potentialities for vibrissa formation, but Hiraiwa[4] obtained one vibrissa in a graft from an 11-day rat embryo to the chick chorioallantois. The present studies of upper lip explants merely show that in the mouse embryo, this skin has the power of complete follicle differentiation by at least 14 days.

Danforth[9] claimed that, since there was a continuous series between pelage hair follicles and vibrissa follicles,[20] the two types of appendage were homologous, and the differences between them were due to different influences acting upon them in organogenesis. The observations on tissue cultures by Murray,[12] and those in the present paper, do not lend much support to Danforth's statement, although it is doubtless true that all follicles are homologous in a general sense. The follicles which develop in rows on the skin of the upper lip in the explants differ from those of pelage hairs in the earliest stage. The former are always larger, and finally they achieve a greater length. There is a constriction above the papilla which gives to each vibrissa follicle the hour-glass shape absent in other follicles. A very thick connective tissue sheath forms around the follicle. Although a quantitative study has not been made, it seems that the differentiation of vibrissa follicles is faster than that of pelage follicles, *in vitro* as well as *in vivo*. Even the gradients within each row in the time of development, and in the size of vibrissa follicles, are maintained in cultivation. These differences in behavior between the two follicle types *in vitro* suggest that such features are not primarily due to blood circulation or nervous stimulation, since both are absent from cultures, to mechanical forces, which are very abnormal in cultures, or to the effect of any tissue other than the dermis and epidermis of a particular body region. One must conclude that any factors in organogenesis which might account for these differences between the follicle types must have operated on the skin before the morphological differentiation of follicles. Other characteristics of vibrissa follicles, such as blood sinuses, special nerve endings, and muscle attachments,[7] do not form in explants and probably require influences outside the skin for their development.

In the mouse, the vibrissae are from the beginning much larger than pelage hairs. In explants, although the vibrissa follicles were the larger, the vibrissae themselves were about the same thickness as the other hairs. The rates of growth in length were similar in the two follicle types. It was suggested previously[13] that the small size of pelage hairs in explants was due to the lack of blood circulation; and the equally small size of vibrissae formed in explants, where their special blood supply is lacking, lends further support to this view. It is noteworthy that, in both follicle types *in vitro*, the histological detail was normal and the other follicle layers were of dimensions similar to those *in vivo*. Apparently, it was only the quantity of keratin produced which was affected by the absence of circulation.

Summary

The body skin of embryonic mice produced normal hair follicles and hairs when cultivated *in vitro*. Follicles which appeared in the skin in explants were arranged in the normal pattern, and those of one generation developed synchronously. These and other features were best seen by the continuous study of living explants under the microscope. Photomicrographic records illustrated the changes from day to day.

The skin of the upper lip of embryonic mice produced characteristically shaped vibrissa follicles and small keratinized hairs *in vitro*. The changes in shape of the dermal papilla and the formation of follicle layers were readily observed in the living explants. A thick fibrous connective tissue sheath was formed around each follicle, but there were no blood sinuses.

Tissue culture has added to the information obtained by grafting and other techniques about the histogenesis of hair follicles. In the mouse embryo, at least as early as 12 days, the dermis and epidermis together have the capacity to form follicles of pelage hairs. Fully differentiated follicles and hairs may be formed from these tissues *in vitro*, and vibrissae may be formed in skin taken from the upper lip at 14 days. The main qualitative differences between vibrissae and pelage hair follicles were already determined in the skin before the follicles appeared, and they were maintained in cultivation despite the uniform environment.

Bibliography

1. Trotter, M. & H. L. Dawson. 1932. The direction of hair after rotation of skin in the newborn albino rat: a second experiment on hair slope. Anat. Rec. **53:** 19.
2. Butcher, E. O. 1946. Hair growth and sebaceous glands in skin transplanted under the skin and into the peritoneal cavity in the rat. Anat. Rec. **96:** 101.
3. Reed, S. C. 1938. Determination of hair structure. I. The production of waved hair from genetically non-waved cells. J. Exp. Zool. **79:** 347.

4. Hiraiwa, Y. K. 1927. Studies on grafts of embryonic tissues of the rat on the chorio-allantoic membrane of the chick. I. Differentiation of ectodermal derivatives. J. Exp. Zool. **49:** 441.
5. Rawles, M. E. 1947. Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zoöl. **20:** 248.
6. Medawar, P. B. 1944. The behaviour and fate of skin autografts and skin homografts in rabbits. J. Anat., Lond. **78:** 176.
7. Vincent, S. B. 1913. The tactile hair of the white rat. J. Comp. Neurol. **23:** 1.
8. Szymonowicz, W. 1936. Comparative examination of the innervation of the sinus hair in mammals. 1. Z. ges. Anat. 1. Z. Anat. EntwGesch. **105:** 459.
9. Danforth, C. H. 1925. Hair in its relation to questions of homology and phylogeny. Amer. J. Anat. **36:** 47.
10. Strangeways, D. H. 1931. The growth of hair *in vitro*. Arch. exp. Zellforsch. **11:** 344.
11. Murray, M. R. 1933a. Development of the hair follicle and hair *in vitro*. Anat. Rec. **57** (suppl.): 74.
12. Murray, M. R. 1933b. Unpublished observations.
13. Hardy, M. H. 1949. The development of mouse hair *in vitro* with some observations on pigmentation. J. Anat., Lond. **83:** 364.
14. Noback, C. R. 1951. Morphology and phylogeny of hair in mammals. Ann. N. Y. Acad. Sci. **53**(3):476.
15. Grüneberg, H. 1943. The development of some external features in mouse embryos. J. Hered. **34:** 88.
16. Lillie, F. R. & H. Wang. 1944. Physiology of development of the feather. VII. An experimental study of induction. Physiol. Zoöl. **17:** 1.
17. Miszurski, B. 1937. Researches on the keratinization of the epithelium in tissue cultures. Arch. exp. Zellforsch. **20:** 122.
18. Waterman, A. J. 1940. Heterotransplants of embryonic mouse tissues. Growth, Ithaca **4:** 33.
19. Reed, S. C. & A. Alley. 1939. The production of pigment in grafts of mouse skin grown on the chorio-allantois of white Leghorn chicks. Anat. Rec. **73:** 257.
20. Botezat, E. 1897. Die Nervendigungen an den Tasthaaren von Säugethieren. Arch. mikr. Anat. **50:** 142.

REGENERATION AND RATE OF GROWTH OF HAIRS IN MAN*

By Raymond J. Myers and James B. Hamilton
Department of Zoology, Colgate University, Hamilton, New York; Department of Anatomy, State University Medical Center at New York City, Brooklyn, New York

The following data represent a preliminary report upon work in progress regarding (1) the number of days required for regeneration of hairs in selected areas of the human body, (2) the rate of growth of hairs in these areas, and (3) the influence of age and sex upon the regenerative interval and the rate of growth.

Materials and Methods

The regions selected for study included two on the scalp, the crown and a supraear site, which lay 5 cm. superior to the auricle along a coronal line drawn between the external auditory meatuses; chin (in males only); axilla; thigh; and eyebrow.

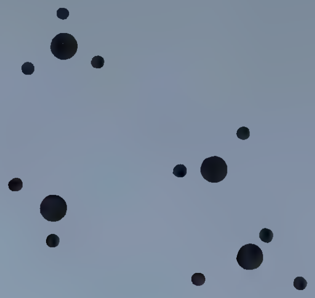


FIGURE 1.

The position of orifices of hair follicles was charted with reference to one to four small, permanent tattoos which were made in the skin with India ink. The tattoos were judged to be permanent if the ink could still be seen 48 hours after tattooing. At this time, the position of the tattoos and the orifices of follicles containing terminal hairs was drawn to scale as illustrated in FIGURE 1. Areas chosen for tattooing contained 6 to 12 hairs. The separately identified hairs were then plucked and preserved for microscopical study. A few hairs were observed to break upon plucking. If the stub could not be pulled immediately, it was removed after an interval of 7 days. Plucked areas were observed at weekly intervals until the regeneration of hairs had been completed.

A hair was considered to have regenerated when its tip projected above the skin. The time required for regeneration of a hair was calculated upon the number of days between plucking of an old hair and the appearance of a new hair above the skin, as observed at intervals of 7 days. If a new

---

*This work was supported in part by grants RG 1036 and H 397 from the U. S. Public Health Service. The authors are indebted to the staff and subjects at Kings Park State Hospital, Rome State School, and Utica State Hospital.

hair was observed to be growing parallel to, but beneath, the outer layers of the epithelium, the hair was probed free. This was then counted as its emergence date.

Calculations were based upon regeneration of hairs in 30, 60, and 90 per cent of the follicles from which hairs had been plucked. In the following data, the analysis is restricted to the interval needed for regeneration of hairs in 90 per cent of the follicles, since the results appear to be less variable than those for the appearance of hairs in 60 or 30 per cent of the follicles. The interval required for regeneration in 100 per cent of the follicles was not employed, because hairs did not return in all follicles during the period of study.

The length of newly-erupted hairs was measured to the nearest 0.5 mm. at weekly intervals in the subjects of Series A and biweekly in subjects of Series B. Thus, the measurements of the rate of growth were carried out in all hairs, beginning at the same stage in their cycle, upon emergence above the surface of the skin.

TABLE 1

SUMMARY OF SUBJECTS IN SERIES A AND B ACCORDING TO AGE AND SEX

Groups	*Late childhood*	*Young mature*	*Old mature*	*Old age*	*Senescent*	*Total no. of subjects*
Series A						
Males	11	21	44, 45	65		5
Females	11	28, 30	44, 52	66, 64		7
Series B						
Males	9, 9	25, 25	44, 45	67, 68	75, 77	21
	10, 10	26, 26	44, 46	68, 71, 74	80, 84	
Females	9, 9, 9	20	41	66, 69	75, 77	21
	10, 10	25, 25	42, 45	70, 71, 73	78, 82, 84	

The average daily rate of growth of all hairs is based upon measurements taken during the period of active growth of the hair, and not during the resting period between the end of the phase of actual growth and the sloughing of the hair. The growth phase was completed in all hairs in the subjects in Series A, except for some hairs of the scalp in women, which were still growing after the one and one-half years of observation.

The data for hairs of the eyebrows may not be representative since, in the site selected for study, only small hairs were present. Similarly, the values for thigh hairs in women can be considered only as suggestive, because the sparseness of hairs in this region in older women limited the number of hairs that were available for study.

In men, the hairs on the scalp and chin had to be clipped periodically, in order to avoid their unintentional loss when the hair of the head was cut or the beard shaved. In the women, clipping was not necessary, but for ease of measurement, the length of the hairs was kept between 10 and 25 mm.

The subjects were 54 in number and were residents of New York State institutions for mental health. Hair growth is normal in such subjects who are in good physical health, as shown by Trotter (1924) and confirmed in unpublished data obtained by one of us (J. B. H.). The advantages of using institutional patients include: (a) a uniform environment and routine of life for all subjects, and (b) ease of study, since these subjects are always available and do not object to minor changes in hair coiffure or physical appearance.

The range of ages was 9 to 84 years (TABLE 1). Subjects were classified in 5 age groups. The sexually immature children consisted of 5 boys and 6 girls, 9 to 11 years old, none of whom had large, pigmented, or kinky pubic hairs when the study was begun. The young mature adults included 5 males and 5 females, between 21 and 30 years of age. The third group, described as old mature adults, included 6 males and 5 females, aged 41 to 52. The old-age adults were 6 males and 7 females, 64 to 74 years old. The fifth group, made up of senescent individuals, included 4 males and 5 females, aged 75 to 84.

TABLE 2
NUMBER OF DAYS REQUIRED FOR REGENERATION OF HAIRS IN 90 PER CENT OF THE FOLLICLES FROM WHICH HAIR HAD BEEN PREVIOUSLY PLUCKED
(ALL AGES AND BOTH SEXES)
Series A

Region of body	*Crown of scalp*	*Supraear region of scalp*	*Chin*	*Axilla*	*Thigh*	*Eyebrow*
Regenerative interval (No. of days)	129	117	92	123	121	64
No. of hairs	141	140	50	107	62	107
No. of subjects	12	12	4	10	6	12

TABLE 3
NUMBER OF DAYS REQUIRED FOR REGENERATION OF HAIRS IN 90 PER CENT OF FOLLICLES FROM WHICH HAIR HAD BEEN PREVIOUSLY PLUCKED IN MALES AND FEMALES (IRRESPECTIVE OF AGE)
Series A

Area	*Crown*		*Supraear*		*Chin*	*Axilla*		*Thigh*		*Eyebrow*	
Sex	M	F	M	F	M	M	F	M	F	M	F
Regenerative interval (No. of days)	110	147	90	144	92	144	102	157	84	66	61
No. of hairs	57	84	56	84	50	42	65	45	17	46	61
No. of subjects	5	7	5	7	4	4	6	4	2	5	7

Series A consisted of 12 subjects in one institution in whom the rate of hair growth was studied for one and one-half years between 1946 and 1948. In Series B, the rate of hair growth was investigated for 60 to 90 days, during 1949 and 1950, in 42 subjects who lived in another institution.

Results

Regeneration. These data were obtained from subjects in Series A.

(1) *Time Required for Regeneration of Hairs in Different Regions of the Body* (TABLE 2). The average number of days required for regeneration of hairs in 90 per cent of the follicles was largest in the scalp, axilla and thigh, less in the chin, and much less in the eyebrow.

TABLE 4

NUMBER OF DAYS REQUIRED FOR REGENERATION OF HAIRS IN 90 PER CENT OF FOLLICLES FROM WHICH HAIR HAD BEEN PREVIOUSLY PLUCKED (IRRESPECTIVE OF SEX)

Series A

Area	*Crown*				*Supraear*				*Chin*			
Age of subjects (in years)	11	21–30	45–52	64–66	11	21–30	45–52	64–66	11	21–30	45–52	64–66
Regenerative interval (No. of days)	134	138	112	139	138	125	124	103		71	86	127
No. of hairs	21	39	48	33	25	39	42	34		14	25	11
No. of subjects	2	3	4	3	2	3	4	3		1	2	1
Area	*Axilla*				*Thigh*				*Eyebrow*			
Age of subjects (in years)	11	21–30	45–52	64–66	11	21–30	45–52	64–66	11	21–30	45–52	64–66
Regenerative interval (No. of days)		98	125	139	77	88	141	240	58	65	56	73
No. of hairs		34	45	28	11	18	24	9	16	29	34	28
No. of subjects		3	4	3	1	2	2	1	2	3	4	3

TABLE 5

AVERAGE DAILY RATE OF GROWTH OF HAIRS IN DIFFERENT AREAS AS CALCULATED FOR SUBJECTS OF ALL AGES AND BOTH SEXES

Series A

Regions	*Crown of scalp*	*Supraear region of scalp*	*Chin*	*Axilla*	*Thigh*	*Eyebrow*
Aver. daily growth (mm.)	0.35	0.35	0.38	0.30	0.20	0.16
No. of hairs	118	109	46	84	47	80
No. of subjects	12	12	4	10	6	12
Series B						
Aver. daily growth (mm.)		0.32		0.32		
No. of hairs		302		118		
No. of subjects		42		26		

(2) *Influence of Sex* (TABLE 3). Hairs of the scalp, in both the crown and the supraear regions, regenerated more rapidly in males (an average of 110 and 90 days) than in females (147 and 144 days). Hairs of the axilla regenerated more rapidly in females (102 days) than in males (144 days). Hairs of the eyebrow regenerated at about the same time in males and females.

(3) *Relationship to Lifespan and Rate of Growth of Hairs.* Unpublished

data show that the lifespan of hairs of the crown and supraear, as calculated in terms of the average number of days between emergence of a hair above the skin and sloughing of the hair, was longer in females (over 20 months) than in males (about 16 months). Thus, those hairs which attain the greatest length regenerate at a slow rate and, as described below, grow at the most rapid rate.

In the axilla there was no relationship between the time required for regeneration and the rate of growth. Regeneration was most rapid in females, whereas the rate of growth was most rapid in males. The lifespan of axillary hairs was approximately the same in both sexes (202 days in males, 204 days in females).

TABLE 6

AVERAGE DAILY RATE OF GROWTH OF HAIRS IN DIFFERENT AREAS AS CALCULATED SEPARATELY FOR MALE AND FEMALE SUBJECTS IN SERIES A (IRRESPECTIVE OF AGE)

Region	*Crown of scalp*		*Supraear region of scalp*		*Chin*		*Axilla*		*Thigh*		*Eyebrow*	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Aver. daily growth (mm.)	0.34	0.36	0.34	0.36	0.38		0.31	0.29	0.22	0.12	0.15	0.16
No. of hairs	46	72	44	65	46		35	49	31	16	28	52
No. of subjects	5	7	5	7	4		4	6	4	2	5	7

TABLE 7

AVERAGE DAILY RATE OF GROWTH OF HAIRS IN DIFFERENT AREAS AS CALCULATED SEPARATELY FOR MALE AND FEMALE SUBJECTS IN SERIES B (IRRESPECTIVE OF AGE)

Region	*Supraear region of scalp*		*Axilla*	
Sex	M	F	M	F
Aver. daily growth (mm.)	0.31	0.34	0.33	0.30
No. of hairs	152	150	76	42
No. of subjects	21	21	16	10

(4) *Influence of Age* (TABLE 4). In all age groups the hairs of the scalp regenerated at a slow rate.

Hairs of the chin, axilla, and thigh required the shortest interval for regeneration in the youngest groups and a progressively longer interval with increasing age. These data for axillary hair may possibly be related to the progressive decrease with age in the growth of this secondary sexual character (Hamilton, page 585 in this monograph), and in the titers of urinary ketosteroids (Hamilton and Hamilton, 1948) and androgens (Hamilton, unpublished data). These titers serve as indices of the quantity of substances that promote growth of axillary hairs.

Age had little influence upon the rate of regeneration of hairs in the eyebrow.

Rate of Hair Growth. (1) *Regional Differences* (TABLE 5). In Series A, the average daily rate of growth was most rapid in hairs of the chin (0.38 mm.) and scalp (0.35 mm.). With the exception of the chin, the average daily rate of growth of hairs which develop as secondary sexual characters was slightly less than that of scalp hairs, amounting to 0.30 mm. in the axilla and 0.20 mm. on the thigh. The slowest rate of growth was the daily average of 0.16 mm. in the eyebrow.

TABLE 8

AVERAGE DAILY RATE OF GROWTH OF HAIRS IN DIFFERENT REGIONS WITH INCREASING AGE OF THE SUBJECTS IN SERIES A (IRRESPECTIVE OF SEX)

Region	*Crown of scalp*				*Supraear region of scalp*			
Age	9–11	21–30	45–52	64–66	9–11	21–30	45–52	64–66
Aver. daily growth (mm.)	0.41	0.30	0.34	0.32	0.37	0.32	0.36	0.31
No. of hairs	14	33	42	29	18	33	24	34
No. of subjects	2	3	4	3	2	3	4	3

Region	*Axilla*				*Thigh*			
Age	9–11	21–30	45–52	64–66	9–11	21–30	45–52	64–66
Aver. daily growth (mm.)		0.36	0.33	0.24	0.13	0.16	0.25	0.19
No. of hairs		27	37	20	10	18	17	2
No. of subjects		3	4	3	1	2	2	1

Region	*Chin*				*Eyebrow*			
Age	9–11	21–30	45–52	64–66	9–11	21–30	45–52	64–66
Aver. daily growth (mm.)		0.42	0.37	0.37	0.14	0.14	0.16	0.16
No. of hairs		8	26	12	8	24		23
No. of subjects		1	2	1	2	3		3

TABLE 9

AVERAGE DAILY RATE OF GROWTH OF HAIRS IN DIFFERENT REGIONS WITH INCREASING AGE OF THE SUBJECTS IN SERIES B (IRRESPECTIVE OF SEX)

Region	*Supraear*					*Axilla*				
Age	9–11	20–26	41–46	66–74	75–84	9–11	20–26	41–46	66–74	75–84
Aver. daily growth (mm.)	0.31	0.35	0.34	0.32	0.30		0.35	0.30	0.31	0.30
No. of hairs	64	52	39	71	66		46	36	23	13
No. of subjects	9	7	7	10	9		7	7	6	6

In the 42 subjects in Series B, the average daily rate of growth was 0.32 mm. for both supraear and axillary hairs, which approximates the values observed in the smaller number of subjects in Series A.

(2) *Influence of Sex* (TABLES 6 and 7). The growth rate of hair in both crown and supraear regions of the scalp was slightly higher in females than in males in both Series A (0.36 *vs.* 0.34 mm.) and Series B (0.34 *vs.* 0.31 mm.). The rate of growth of axillary hairs was slightly higher in males than in females in both Series A (0.31 *vs.* 0.29 mm.) and Series B (0.33 *vs.* 0.30 mm.).

Hairs of the eyebrow grew at about the same rate in males (0.15) and females (0.16 mm.).

(3) *Influence of age* (TABLES 8 and 9). The growth rate of axillary hair decreased markedly with age. Reduction in the rate of growth of beard hair with age was observed in Series A, but this phenomenon was not studied in Series B.

Summary and Conclusions

1. The average number of days required for regeneration of hairs in 90 per cent of the follicles from which hairs were plucked was: 129 in the crown of the scalp, 123 in the axilla, 121 in the thigh, 117 in the supraear region of the scalp, 92 in the chin, and 64 in the eyebrow. This slow rate of regeneration in scalp hairs was observed in all age groups.
2. Hairs of the scalp regenerated more rapidly in males than in females, whereas, hairs of the axilla and thigh hairs regenerated more rapidly in females than in males.
3. The rate of growth of hairs, in order of decreasing rapidity, was as follows: chin (0.38 mm. per day), crown of scalp (0.35), supraear region of scalp (0.35), axilla (0.30), thigh (0.20), and eyebrow (0.16). Thus, in the regions under study, the long hairs, those of scalp and chin, have the fastest rate of growth, whereas the short hairs, as in the eyebrow, have the slowest rate. Moreover, it is in these regions, in which hairs are long and grow at the fastest rate, that the hairs require the longest interval for regeneration.
4. The rate of growth of hairs of the scalp is faster in females than in males, even in the supraear region, which is not subject to baldness. In the axilla, the daily growth rate of hairs is more rapid in males than in females. A decrease in growth rate with age was observed in axillary hairs.

Bibliography

DANFORTH, C. H. 1925. Hair, with Special Reference to Hypertrichosis: 152. A. M. A. Chicago.

GIOVANNINI, S. 1890. De la régénération des poils aprés l'epilation. Arch. f. mikrosc. Anat. **36:** 528–564.

HAMILTON, H. & J. HAMILTON. 1948. Ageing in apparently normal men. I. Urinary titers of ketosteroids and of alpha-hydroxy and beta-hydroxy ketosteroids. J. Clin. Endocrin. **8:** 433–452.

HAMILTON, J. B. 1951. Quantitative measurements of a secondary sex character, axillary hair. Ann. N. Y. Acad. Sci. **53**(3): 585.

TROTTER, M. 1923. The resistance of hair to supposed growth stimulants. Arch. Derm. and Syph. **7:** 93–98.

TROTTER, M. 1924. The life cycles of hair in selected regions of the body. Am. J. Phys. Anthrop. **7:** 427–437.

TROTTER, M. 1928. Hair growth and shaving. Anat. Rec. **37:** 373–379.

CHANGES IN MORPHOLOGY OF HAIR DURING CHILDHOOD*

By Oliver H. Duggins† and Mildred Trotter
Department of Anatomy, Washington University, St. Louis, Mo.

The general appearance of hair, as seen by the naked eye, is sufficiently variable to warrant including the type of hair as one of the chief differentiating characteristics of race. Microscopic study of individual characters contributing to the differences in appearance of head hair of a Negro, a Mongol, and a White has shown definite variations in index, size, pigmentation, medullation, and other morphological features. Nevertheless, the range of variation of a given character within a race is not only very wide, but it also overlaps the range of the same character of the other races.

The appearance, or general effect, of heads of hair within a homogeneous group may often be very different, and it is believed that this effect is different for the same head at different ages. Structural characters of human head hair from many different individuals of various ages have been studied. Wynkoop (1929), in studying head hair of a series of 82 individuals (presumably White), found little, if any, correlation between the age of the individual and the diameter of the shaft. She also reported that the type of medulla bears no relation to age of the individual but that there is a correlation between the type of medulla and the size of the hair. The larger hairs are more likely to have continuous medullas, while the smaller hairs have either very scanty or no medullas. In a series of 340 American Whites (Trotter, 1930), there was no significant variation in index between different age periods, but the size of the shaft was found to be significantly less in groups under the age of 10–15 years than in older groups. Gamble and Kirk (1941) examined hair from 37 White persons of varying ages for scale count and found both the means and ranges of the scale counts to be approximately constant for the same individual but variable between individuals. In addition, a single hair was determined to be characteristic of all hairs counted for the individual with respect to mean and nearly always with respect to range of scale counts.

A study of hair from the same individual over a period of years is expected to present evidence from which the magnitude and significance of individual variations, in relation to both physiological and chronological age, may be determined. This is a report of findings in a study of four characters, *i.e.*, index, size of shaft cross section, incidence of medullation, and the scale count, on hair from the same individuals, beginning at birth and extending to 14 years of age. It is expected that the study will be conducted through 25 years of age and that other characters will be investigated as well.

Material and Method

A collection of monthly hair samples starting at birth was begun in 1930. Sixteen American White children are contributors, the last one having been

---

*This investigation was supported (in part) by a research grant from the Viking Fund, Inc., and (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

† In partial fulfillment of the requirements for the degree of Doctor of Philosophy.

born in 1942. The samples were cut close to the scalp in the region of the vertex. The group includes nine girls and seven boys. There are five pairs of siblings, of which one pair are identical twins.

The determination of index was based on the greatest and least diameters of the shaft (least diameter × 100, divided by greatest diameter), taken as close to the proximal end of the sample as was feasible. Fifty hairs, taken at random from each sample at six-month intervals, were measured after sectioning with the Hardy microtome, and, for these, the average index for each year for each subject was determined.

The size or area of a cross section of the hair at the level at which the index was found was determined in mm.[2] by the formula: $\frac{1}{2}$ greatest diameter × $\frac{1}{2}$ least diameter × 3.1416. The samples used for these determinations were used for the determinations of indices (Trotter and Duggins, 1948). The total area of each hundred hairs for each year was recorded (thus, by shifting the decimal point, the average area of one hair may be determined).

The percentage incidence of medullas, of all types, scanty, broken, and continuous, was based on a study of 50 hairs for each year. Twenty-five hairs were chosen at random from samples selected at six-month intervals. For the first seven months, however, all hairs of each sample, ranging in number from 50 to 300, were examined.

The scale counts are recorded in terms of the mean for 100 counts, each count having been made over a distance of 0.16 mm. on one hair, from samples at 12-month intervals. A scale was included in the count, providing its free edge crossed the longitudinal straight line of measurement, which was parallel to the shaft. The free edges of the scales were better defined by the application of a drop of glycerine and alcohol mixture at each end of the shaft, which mixture progresses along the shaft by capillary attraction and brings into relief the free borders of the scales.

Results and Discussion

Graphs, constructed for index, area, scale count, and percentage incidence of medullation according to age, are presented for each individual in FIGURE 1. It may be seen that the index drops sharply during the first two years of life, after which there is irregularity, with little consistency in trend for most individuals. Subject H is an exception, showing a slight rise in index during the first two years instead of a drop. Subject O is also an exception, showing no change at all.

The size or area of the cross section of hairs shows a rapid and uniform increase during the first three or four years of life, a less rapid and less uniform increase during the next six years, and then either a slow increase or a levelling off. Subject C is exceptional in presenting hair which continues to increase in size at a more rapid rate than the other subjects.

The percentage of hairs presenting medullas (scanty, broken, or continuous) is very low at birth and increases markedly at some time during the first seven months of life. During the second half of the first year, the percentage incidence of medullas continues to increase in three indi-

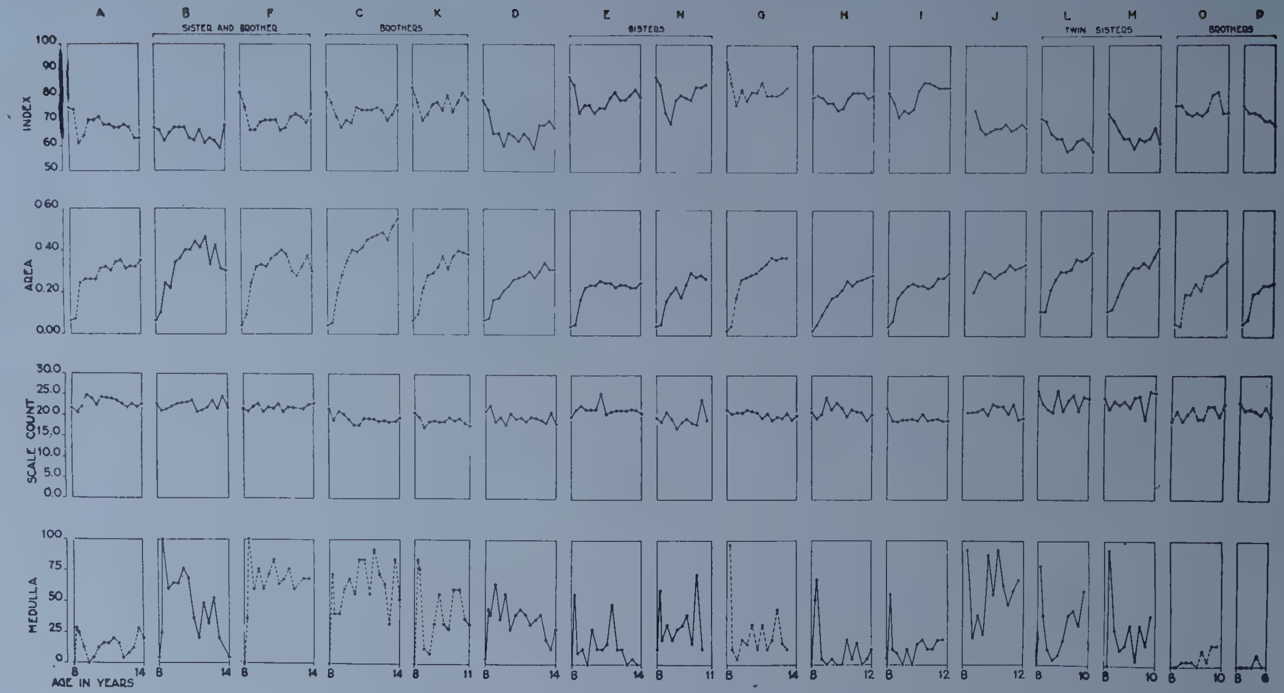


FIGURE 1. Individual graphs according to age for index, area (in mm.²), scale count (per 0.16 mm.), and percentage incidence of medullas. Broken lines with solid circles, boys; continuous lines with open circles, girls.

viduals, *i.e.*, in Subjects B and F, who are brothers, and in Subject H. Birth samples are not available for the identical twins, Subjects L and M. The trends for Subjects L and M are in direct contrast to each other for the period from seven months to one year, rising for M and dropping for L, after which they follow a similar pattern. In Subjects O and P, who are brothers, no hairs presenting medullas were found until after one year of age, and, in P, not until after three years. In the remaining nine subjects, the percentage incidence of medullas drops during the second half of the first year. Between one and two years, the changes shown by the medulla incidence are variable from individual to individual (nine subjects show a drop, five a rise, and two remain the same). After two years of age, there is great irregularity, with a slight tendency for the incidence of medullas to increase up to nine years, but in only three subjects does it reach as high an incidence as was reached during the first year. After nine years, the incidence continues to be variable.

The number of scales within a distance of 0.16 mm. along the shaft shows a drop during the first year in all but three of the 15 children for whom samples are available. In the three exceptions, D, E, and O, the scale count increases during the first year. Upon analysis, however, none of these changes are found to be statistically significant. Following the first year, irregularities are apparent for most of the 16 children, with no particular trend. An analysis of variance indicates a significant individual difference in scale count but an insignificant age difference.

Due to the limited number of subjects involved in this investigation, any comparison of characters with regard to sex necessarily would be inconclusive. Based upon the nine girls and seven boys who are subjects for this study, however, it is observed that there is no difference in index for the first three years. Following the third year, five of the nine girls maintain a lower index than the remaining four girls, whose indices are similar in range to those of the boys. When the curves for size of the girls' hair are superimposed upon the curves for size of the boys' hair, the area of the hair of more than half of the girls, between the second and eighth years, is found to be less than of the hair of the boys. A comparison of the total number of hairs containing medullas shows that the boys lag slightly behind the girls for the first two years, parallel the girls rather closely between the second and fifth years, and then continue slightly above the girls up to 14 years of age. With regard to the type of medulla, however, the boys are found to exhibit more than three times as many broken and continuous medullas as the girls. No correlation is noted between scale count and sex.

A comparison of these characters with regard to the siblings reveals some similarities. The curves for size and index are observed to be more closely alike for the related subjects than for the unrelated subjects, with the greatest similarity existing between the identical twins. Similarity in medulla incidence is quite marked for several of the siblings, but is found to be no more pronounced for them than for several of the unrelated subjects. Only two of the subjects exhibit no medullas during the first seven months of life. These are O and P, who are brothers. No correlation is present between the scale counts of related subjects.

The composite curves for each character (FIGURE 2) are much smoother, as might be expected, than the individual curves. They show that the period from birth to the second year is the period during which the most marked changes occur. Also, the changes in this period are most consistent from individual to individual: for index, there is a drop; for area, there is a rise; for incidence of medullas, there is a rise, followed by a drop; and, for the number of scales, there is a very slight drop during the first year, with no change during the second year. Thus, the shape of the cross section of the hair shaft changes from a more nearly round shape to a more nearly ovoid outline; the area of the cross section is increased or the hair becomes coarser; the number of hairs presenting medullas increases markedly during the first year and drops during the second year, but does not reach as low an incidence as obtained at birth; and the number of scales decreases during the first year to a slight extent. After the second year, the index, area, and medullation curves all rise, whereas the scale count maintains a level. The last few years of this 14-year age span of the composite curves present some irregularities which may be attributed, at least in part, to the fact that only half of the 16 children have reached 14 years of age and the other half are of varying ages under 14 years.

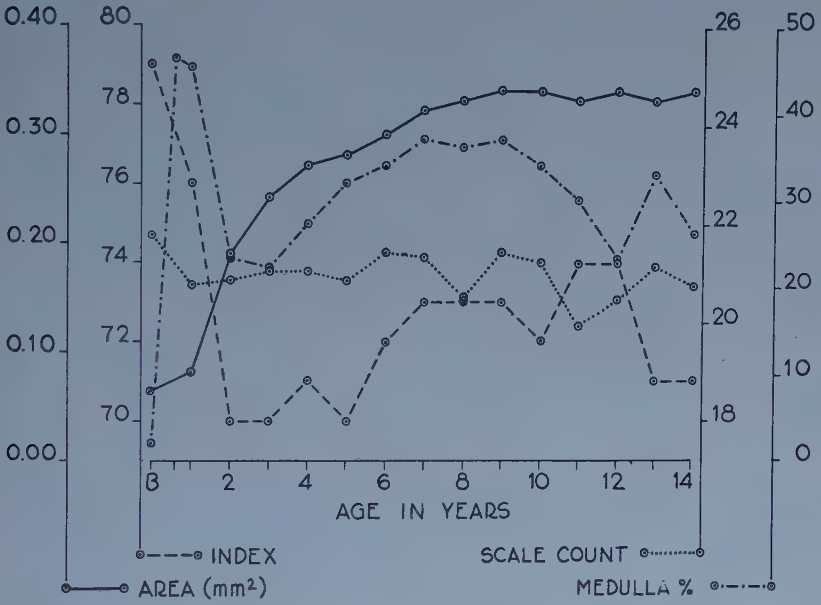


FIGURE 2. Composite graphs for all subjects for index, area, scale count, and medulla incidence according to age.

In spite of the great individual fluctuation and the lack of uniformity in fluctuation between individuals, there are certain changes, with relation to chronological age, which cannot be denied. Although the composite curve of index exaggerates the degree of drop during the first two years of life, there must be an actual change in shape over and above the range of variation for either the individual or the group, since the drop occurs consistently (or nearly so) for each individual. The fluctuations thereafter appear to be without significance. Coarsening of hair, particularly in the early years of life, can be observed with the naked eye. Thus, the increase in area with age was expected. The medulla incidence is extremely variable from year to year and from individual to individual, but the most extreme change occurs for each child (excepting O and P, who are brothers) during the first two years. The nature of this change, an increase during the first year followed by a drop during the second year, is incompatible with the direct relationship between medulla incidence and size of shaft found in older subjects (Wynkoop, 1929). Since the size of the shaft shows a consistent increase during the early years, it would be expected that the medulla incidence would also increase.

The variations from year to year, seen for each character in each of the 16 individuals, are difficult to explain. It has been pointed out that a hair varies in its size and form along the length of its shaft. However, these variations are known to be small (Kneberg, 1935). Likewise, it has been observed that there is a wide degree of variation, not only between hairs from different regions of the same head, but also among adjacent hairs from the same region. Error in observation or measurement is reduced to a minimum, since all observations in any one category of this study were made by the same individual.

It is suggested that the chronological age changes in hair are probably due more to the type of hair than to any other factor or group of factors. During the fetal stage of an individual, the hair which develops over the body, including the scalp, is called "lanugo." This is very fine in character and, except for size, does not differ, so far as is known, from larger hairs. Although possibly one or more generations of the lanugo are shed before birth, many hairs may persist after birth and are believed to be succeeded by vellus during the first year of life. "Vellus" is described as the fine hair of postnatal life. As the hairs in succeeding generations become coarser, they are classified as intermediate, and the still larger ones as terminal hairs. These categories are ill-defined but, in general, it is understood that, in addition to the increased thickness of the shaft, there is also increased pigmentation and a tendency toward continuous medullas. Vellus is persistent over many parts of the body throughout life, and, on the head, all three types, vellus, intermediate, and terminal hairs, are found. For the head, Danforth (1925) has estimated that, in the adult, six to 25 per cent of the total number is of the vellus type. Whereas there is some evidence that the hairs produced by a given follicle may show continuous, though slight, change throughout life, it is true that the greatest change, at least for the head hair, is effected soon after birth. The transition from lanugo to terminal hair for the large majority of the hairs of the head occurs early in postnatal life and may account for the changes observed in index, area, scale count, and incidence of medullation in this study. The persistence of some vellus among hairs of the head, all of which appear, to the naked eye, to be terminal, together with the fact that the successive samples from the same child were not from the same follicles, may account to a great extent for the yearly fluctuations in the individual curves.

Summary

The index, area, percentage incidence of medullation, and the number of scales of hair from the vertex have been determined yearly from birth to 14 years of age for 16 White children.

Certain changes were found to be associated with age:

The index drops sharply during the first two years of life, after which there is irregularity, with little consistency of trend.

The size or area shows a rapid and uniform increase during the first three or four years of life, a less rapid and less uniform increase during the next six years, and then either a slow increase or a levelling off.

The percentage incidence of medullas is very low at birth and increases rapidly during the first seven months of life. After seven months, extending to the second year, there is a decrease, followed by great irregularity, but with a general tendency to rise.

The scale count drops slightly during the first year for most subjects, after which there is no particular trend. An analysis of variance indicates a significant difference in the means of the scale counts of the individuals but an insignificant age difference.

The range in index and size for any one individual is almost as great as for the entire group. Medullation is sufficiently characteristic in a number of individuals to be significant. Of these four characters, the scale count shows the least range of variation.

References

Danforth, C. H. 1925. Hair. Am. Med. Assoc. Chicago.

Gamble, L. H. & P. L. Kirk. 1941. Human hair studies. II. Scale counts. J. Crim. Law & Criminology **31:** 627–636.

Kneberg, M. 1935. Improved technique for hair examination. Am. J. Phys. Anthrop. **20:** 51–67.

Trotter, M. 1930. The form, size and color of head hair in American Whites. Am. J. Phys. Anthrop. **14:** 433–445.

Trotter, M. & O. H. Duggins. 1948. Age changes in head hair from birth to maturity. I. Index and size of hair of children. Am. J. Phys. Anthrop., n.s. **6:** 489–506.

Wynkoop, E. M. 1929. A study of the age correlations of the cuticular scales, medullas, and shaft diameters of human head hair. Am. J. Phys. Anthrop. **13:** 177–188.

THE APPEARANCE OF ADULT PATTERNS OF BODY HAIR IN MAN

By Earle L. Reynolds

Department of Anthropology, Antioch College, and Physical Growth Department,
Fels Research Institute for the Study of Human Development,
Antioch College, Yellow Springs, Ohio

There are wide individual variations, both in time of appearance and in patterning, of terminal body hair in man. Definitive body hair usually appears at puberty, or before, and may continue throughout life. It is not unusual for a man to have only a few straggling chest hairs at twenty and be well endowed with a hirsute mat at forty. On the other hand, pigmented hair may appear at adolescence and not continue to any degree in later life. It may also appear in some regions of the body only after adulthood is reached, for instance, on the external ear.[1]

The distribution of hair on the body also varies within a wide range. It is a safe bet that most adults will have pubic hair and will not have hair on the palmar surface of the hands,[2] but, between these limits, there are opportunities for a considerable variety of distribution. In addition, the differences between the sexes in the amount and distribution of body hair are well known.[3]

A study of adult patterns of body hair presents, therefore, a number of observational problems and may proceed along several lines of inquiry. For instance, we can observe individual cases throughout the life-cycle, and this is perhaps the least equivocal method. It is a slow and tedious process, however, and there are other ways of approaching the problem. It is possible to work out percentage distributions for the presence of terminal hair in various body regions in adults or study distribution of terminal hair at various age-levels in adolescent children. Information of some importance can also be obtained by a comparison of children with their parents and by assaying resemblances and differences in the distribution patterns of twins and triplets. All of these methods are in use at the Fels Research Institute.

The Institute has been engaged since 1929 in an intensive longitudinal study of the growth and development of about 300 normal white children.[4] Our subjects are being followed from fetal life through maturity, and the oldest children are now 21 years of age. Thus, the study of adult patterns of body hair is barely underway. We have approximately 50 children of each sex who have passed the stage of beginning pubic hair. In addition, we have made examinations and taken photographs on 100 parents of each sex.

Information on body hair is obtained from physical examinations and nude photographs (front, side, back) taken twice yearly. Since the analysis of photographs forms an important part of the study, and since this is a common method of studying hair distribution, it may be of interest to demonstrate some of our photographic materials. It should be mentioned that the quality of the photograph is of great importance in assaying body hair, since a good print will show evidence lost in a poor print.

FIGURE 1 shows the general range of distribution of body hair in our series of adult males. The man with least body hair is compared with two of our more hairy subjects. One of the cases is included because of the interesting combination of well-developed body hair with a rather feminoid body shape. Some studies have indicated a degree of association between body build and hirsutism,[5] and more work in this area should prove fruitful.

Individual patterns of appearance and distribution of body hair are illustrated in FIGURE 2. We have been studying for some time certain differences between early- and late-maturing children,[6] and, as our cases reach adulthood, we will look for possible differences in body hair distribution. FIGURE 2 shows an early-maturing girl, in whom pubic hair and breast development began at 9 years, and axillary hair appeared at 9.5 years. Also shown is a 13.5 year-old girl with a noticeable amount of terminal hair surrounding the areolae of the breasts. Another example of individual variation, on the same plate, is a boy of 5.5 years, with a considerable amount of hair on the lower back. His father, not shown, has a marked amount of hair on shoulders and back.

In a number of cases, we have observed that, when a child shows an unusual pattern of hair distribution, one of the parents is very likely to show the same pattern. For example, one of our girls (not shown) had, at the age of fourteen years, a dense growth of hair on the posterior thigh. Her mother and sister show the same pattern. In another instance, a boy of six years has a marked growth of pigmented hair on his forearm, in a pattern identical to his father.

In general, we have noted a tendency for hirsutism to run in family lines. Parents with a considerable amount of body hair are more likely to have children in whom body hair appears early and luxuriously. FIGURE 3 shows a boy with advanced body hair at 16.5 years and his father, who has a more than average amount of body hair. The mother, not shown, also has a heavy growth of hair on the inner thighs and limbs. Parents are by no means an infallible guide to the degree of body hair an adolescent may be expected to develop, however, as is also shown in FIGURE 3, where a 16.5 year-old boy with a noticeable degree of hair on buttocks and trunk has a father with very little hair in this region.

We have been concerned for some years in the study of the developmental patterns of twins and triplets.[7, 8] In FIGURE 4, the appearance of the body hair in identical triplets is shown, together with the father's pattern. There are a number of slight individual differences in body hair pattern, and these are in line with small differences in general maturation and skeletal development. In another case, not shown, two girls of a triplet set, who are identicals, have a thin line of hair along the linea alba, while the third girl, the sibling, does not have this characteristic. The study of twins and triplets provides a valuable tool in the study of body hair, since minute differences can be observed, and a search can be made for factors associated with these differences.

Of the body regions which have been studied, the pubic area has provided a fairly regular sequence of changes in hair growth, and it has been possible to work out standards of development during adolescence.[9] Fels standards

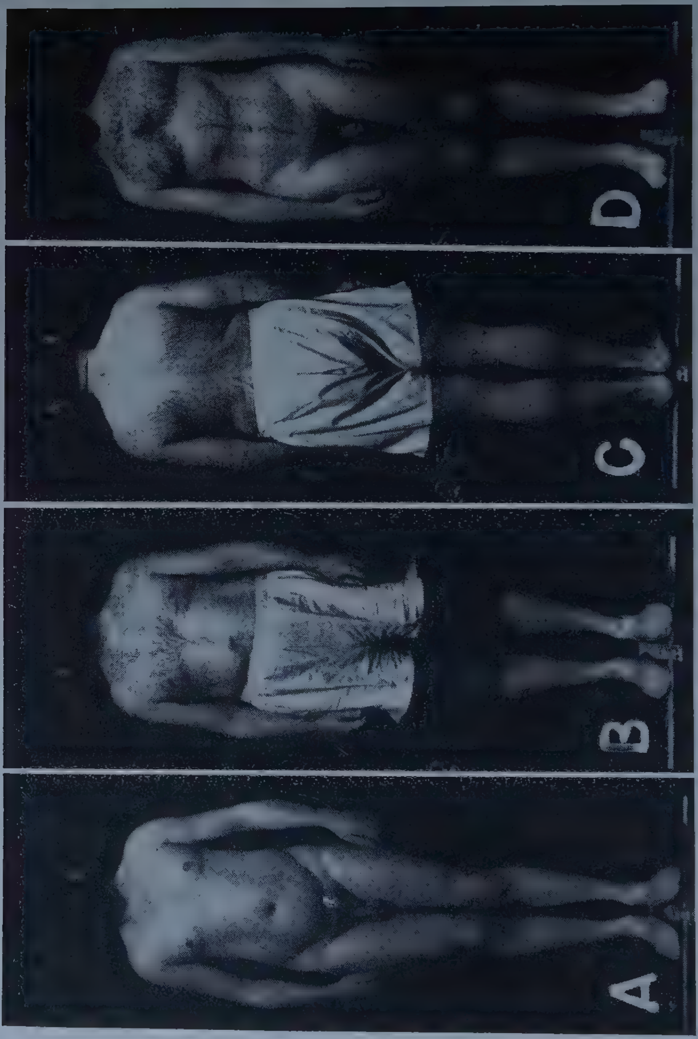


FIGURE 1. Range of body hair in adult males of present series. A—Man with least amount of body hair; B–C, D—men with most body hair. Note feminoid body build of D.

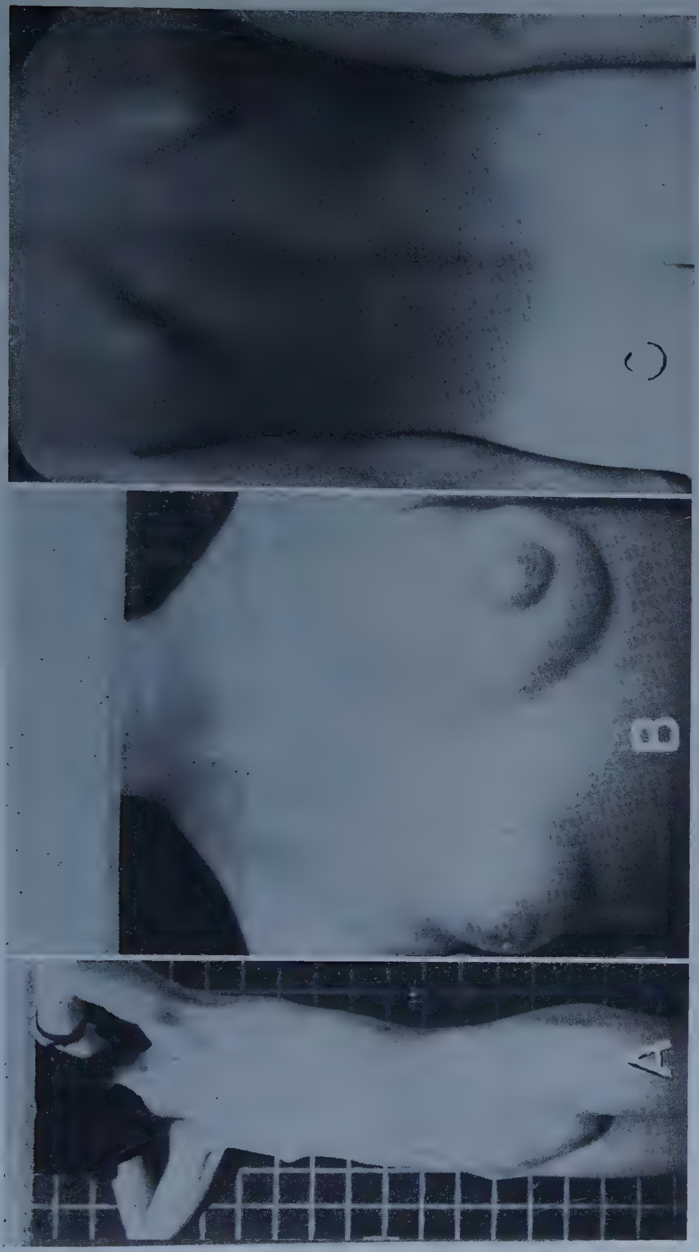


Figure 2. Individual patterns of appearance and distribution of body hair in children. Early maturation (A): pubic and axillary hair in 9-year-old girl; unusual pattern (B): pigmented areolar hair in 13.5-year-old girl; family pattern (C): pigmented hair on lower back of 5.5-year-old boy.

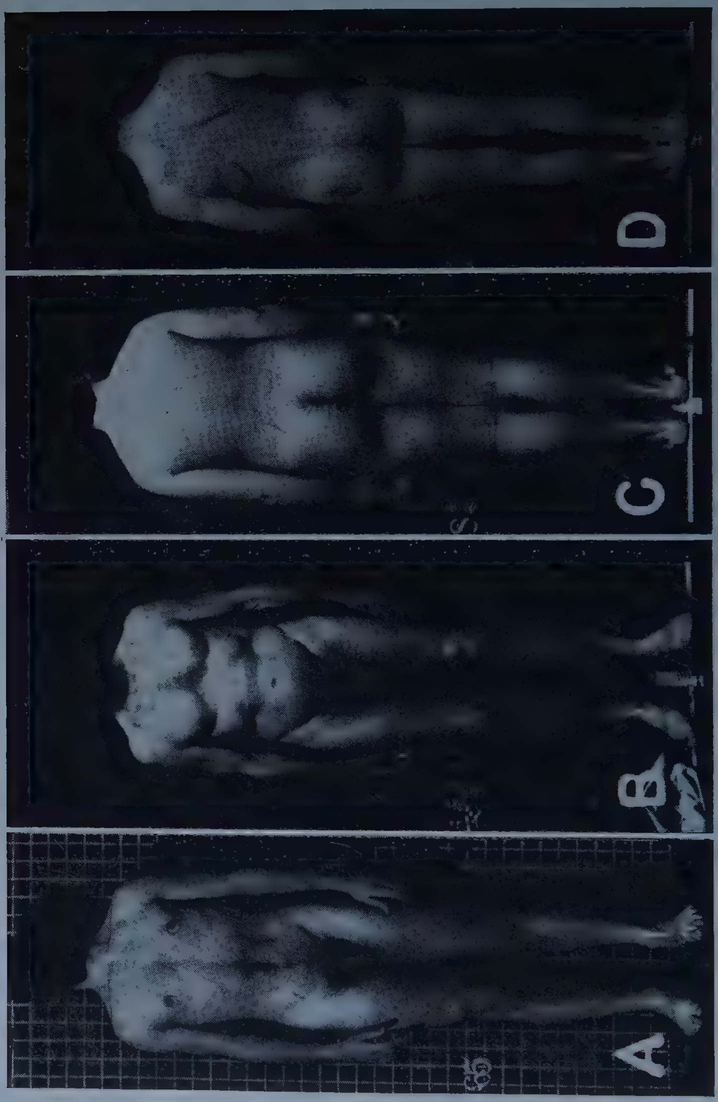


FIGURE 3. Relations between sons and fathers in body hair pattern. Son (A) at 16.5 years with advanced body hair and father (B) with more than average amount. Son (C) with advanced body hair at 16.5 years and father (D) with less than average amount.

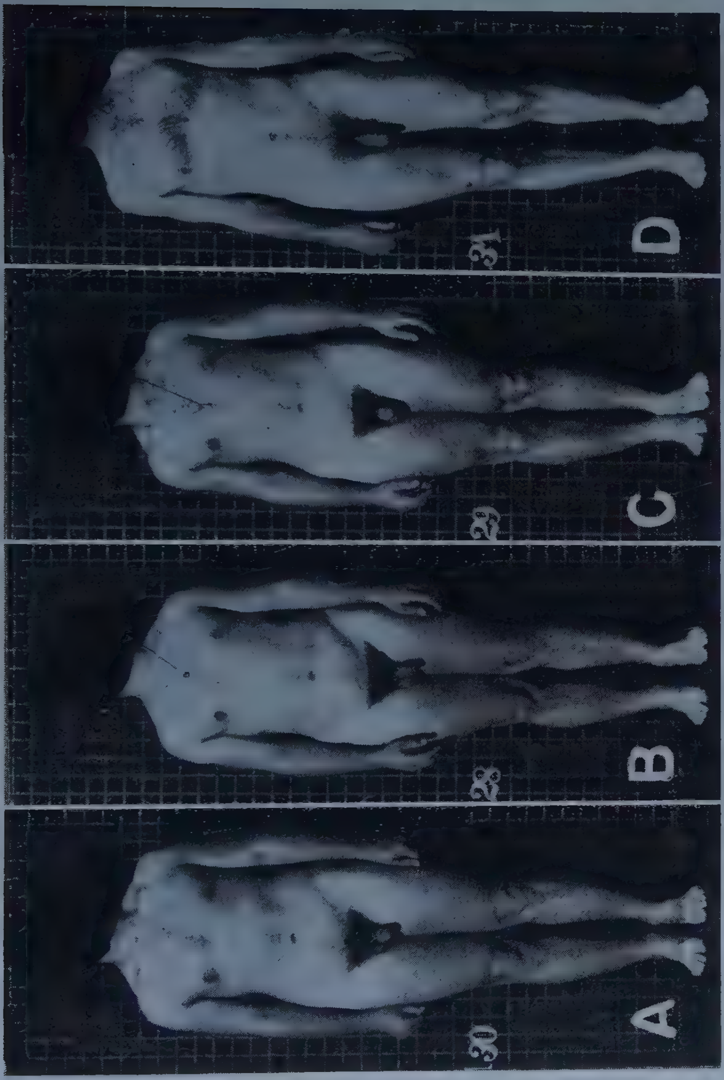


FIGURE 4. Body hair distribution in identical triplets. Triplets (A, B, C) at 21 years and father (D). Note slight individual differences in distribution.

for girls have been published,[10] and those for boys are in preparation. The patterns are similar for the sexes during adolescence, with the girls, of course, reaching the various stages at an earlier age than the boys:

Stage	*Average age, years.* Boys	Girls
1. Infantile. Unpigmented vellus.		
2. First appearance of pigmented hair: Usually straight, sparse; at base of penis in boys, along labia in girls.	12.5	11.0
3. Slight curl, slight spread, usually darker.	13.5	12.0
4. Curled, moderate amount and spread, does not yet extend to thighs.	14.0	12.5
5. The horizontal pattern: profuse, forms an inverse triangle extended to the thighs.	15.5	14.0

Adult patterns of pubic hair distribution have been described by Dupertuis *et al.*,[3] as horizontal, sagittal, acuminate, and disperse. The horizontal, or classically "feminine" pattern (Stage 5 above), predominates in adult females, about 90 per cent having this type, but about 40 per cent of young adult males and 20 per cent of older males also show this so-called feminine pattern. About half the males studied have the acuminate, or classically "masculine" pattern, and there appears to be a shift, with age, toward a larger number of males with a disperse pattern of pubic hair distribution. In both sexes, there is a small scattering of individuals with a sagittal type of distribution. Dupertuis concludes, and rightly, that a simple division of pubic hair distribution into horizontal and acuminate patterns, relating them strictly to feminine and masculine attributes, respectively, is not entirely satisfactory.

Our own study of adolescents and adults is in general agreement with the above findings. The distribution of pubic hair pattern in 50 adult males, average age 38 years, is shown in TABLE 1. About one-fourth show the horizontal pattern and another one-fourth the acuminate pattern. The largest group, 38 per cent, have the disperse pattern of distribution. In our adult females, about 90 per cent have the horizontal pattern.

We have also studied the associations between pubic hair pattern and the amount of hair on thigh and chest, as shown in TABLE 1. The horizontal pattern is associated chiefly with sparse hair on both thigh and chest, the acuminate pattern with heavier thigh hair but with sparse chest hair, and the disperse pattern with heavier hair on both thigh and chest. There appears to be a tendency for a change with age in pubic hair pattern from horizontal through acuminate to disperse. Hair on the upper body comes later in life: of our 18-year-old boys studied, only one in twenty had heavy chest hair, while 30 per cent of our older men showed this characteristic.

In TABLE 2, an attempt has been made to obtain some idea of the sequence of appearance of body hair by determining percentage of boys having hair in various areas at three age-levels. For instance, at 14 years only 14 per cent of our series had hair on the abdomen; at 16 years, 37 per cent had this characteristic; and, by 18 years, 75 per cent had it. In a series of 50 adults, also shown in TABLE 2, abdominal hair was present in 88 per cent. Using this method, we have obtained a tentative ranking of body hair, in time of appearance. In this analysis, facial and head hair have not been considered. The sequence of appearance is: pubic, axillary, anterior leg, posterior leg, posterior thigh, anterior thigh, forearm, abdomen, buttocks, chest, lower back, arm, and shoulder. Obviously, this preliminary listing must be checked as our series of cases increases in size.

TABLE 1

DISTRIBUTION OF PUBIC HAIR IN 50 ADULT MALES, AND RELATIONS OF PUBIC HAIR PATTERN TO MEDIAL THIGH HAIR AND TO CHEST HAIR

Pubic hair pattern	*Sparse hair on thigh*		*Heavy hair on thigh*	
	Hair on chest		*Hair on chest*	
	sparse	*heavy*	*sparse*	*heavy*
	%		%	
Horizontal (28%)	22	0	2	4
Sagittal (6%)	4	0	2	0
Acuminate (28%)	4	4	18	2
Disperse (38%)	4	2	8	24
Totals (100%)	34	6	30	30

TABLE 2

PERCENTAGE OF BOYS HAVING HAIR IN VARIOUS AREAS OF THE BODY AT THREE AGE LEVELS* AND APPROXIMATE RANKING IN ORDER OF APPEARANCE

Area	*14 Yrs.*	*16 Yrs.*	*18 Yrs.*	*Rank*	*Distribution in 50 adults*
Pubic	97	100	100	1	100
Axillary	40	97	100	2	100
Anterior leg	46	90	100	3	88
Posterior leg	38	77	95	4	78
Posterior thigh	35	70	95	5	82
Anterior thigh	30	67	95	6	86
Forearm	14	37	80	7	100
Abdomen	14	37	75	8	88
Buttocks	14	33	50	9	20
Chest	3	7	40	10	74
Lower back	3	7	20	11	33
Arms	0	0	10	12	24
Shoulders	0	0	0	13	9

*No. of cases: 14 yrs., 37; 16 yrs., 30; 18 yrs., 20

The study of adult patterns of body hair distribution is only now underway. The principal needs for further research in this area seem to be: larger series of normal cases followed over longer periods of time; further experimental work on controlled cases; additional types of observation and better methods of classification and measurement; and an intensive study of those factors (hereditary, racial and ethnic, nutritional, endocrine and chemical, and clinical and pathological) associated with, and presumably affecting, the appearance, distribution, and growth of body hair.

Certainly, for the present study, another decade will be needed before a more definitive picture can be drawn. By this time, our adolescents will have emerged into young maturity, and we will have a better idea of the changes taking place in our parent population. In the longitudinal study of human beings, there is no substitute for time.

Bibliography

1. Greulich, W. W., R. I. Dorfman, H. R. Catchpole, C. I. Solomon, & C. S. Culotta. 1942. Somatic and endocrine studies of puberal and adolescent boys. Monograph, Society for Research in Child Development. National Research Council. Washington, D. C.
2. Clark, W. E. Le Gros. 1945. The Tissues of the Body. Clarendon Press. London.
3. Dupertuis, C. W., W. B. Atkinson, & H. Elftman. 1945. Sex differences in pubic hair distribution. Human Biol. **17:** 137.
4. Sontag, L. W. (Ed.) 1946. The Fels Research Institute for the Study of Human Development. Antioch College. Yellow Springs, Ohio.
5. Sheldon, W. H., S. S. Stevens, & W. B. Tucker. 1940. The Varieties of Human Physique. Harper and Brothers. New York.
6. Reynolds, E. L. 1946. Sexual maturation and the growth of fat, muscle and bone in girls. Child Dev. **17:** 121.
7. Sontag, L. W. & E. L. Reynolds. 1944. Ossification sequences in identical triplets. J. Heredity **35:** 57.
8. Reynolds, E. L. & G. Schoen. 1947. Growth patterns of identical triplets from 8 through 18 years. Child Dev. **18:** 130.
9. Stuart, H. C. 1946. Normal growth and development during adolescence. New England J. Med. **234:** 666.
10. Reynolds, E. L. & J. V. Wines. 1948. Individual differences in physical changes associated with adolescence in girls. Amer. J. Dis. Child. **75:** 329.

QUANTITATIVE MEASUREMENT OF A SECONDARY SEX CHARACTER, AXILLARY HAIR*

By James B. Hamilton

Department of Anatomy, State University Medical Center at New York City, Brooklyn, New York

This article, which summarizes data to be published in detail elsewhere, has the following theses: (1) axillary hair is a secondary sex character; (2) this character can be measured quantitatively; (3) these quantitative measurements, which bear a general relationship to titers of urinary ketosteroids and androgens, can be used to assess certain aspects of the endocrine status of individuals; and (4) growth of this character is produced, ordinarily, by secretions of ovaries and testes and not to any large extent by secretions of the adrenal cortices.

It is the further intention (5) to provide data pertaining to growth of axillary hair in males and females at all ages, with the expectation that these data may serve for at least preliminary standards, and (6) to examine some of the factors which influence growth of this secondary sex character.

A relationship of pubic and axillary hair to the endocrine status has been recognized by many investigators, notably Crampton (1908), Koch (1921), Priesel and Wagner (1931), Greulich and associates (1942), Reynolds and Wines (1931), and Kinsell and associates (1946). Definite and easily recognized morphologic changes in pubic hair have been shown to characterize successive physiologic epochs at the time of sexual maturation (Crampton, 1908) and to be accompanied by step-like increments in titers of urinary steroids (Greulich and co-workers, 1942).

Materials and Methods

Subjects consisted of 260 males and 188 females between 15 and 92 years of age. Their range of age is given in TABLE 1.

The nature and the routine of life led by these subjects, many of whom lived in institutions, is described in another article in this monograph,† which deals with the status of the scalp in these same subjects.

Seventy eunuchs and 10 oöphorectomized women were also investigated. Samples of hairs were obtained by shaving the right axilla with a previously unused razor blade held in one of two identical Gillette safety razors. All blades were from a single batch provided by the manufacturer.

Before weighing, shavings of hair were placed in ether to remove fats and then dried in a desiccator. Two other precautions were found to be vital. Hairs had to be separated from sloughed skin, lint, and other debris. The desiccated hairs had to be weighed in such a manner that their hygroscopic properties did not invalidate the measurements.

Three means of calculating the growth of axillary hairs were employed: (1) weight of full-grown mass of axillary hair; (2) weight of the hair grown

---

*This study was supported in part by research grants RG 1036 and H 397 of the U. S. Public Health Service. It is a pleasure to acknowledge the technical aid of Gordon Mestler and the cooperation and helpfulness of subjects and staff at the institutions where this work was carried out.

†P. 562.

per day; and (3) ratio of the average daily rate of growth to the weight of the full-grown mass of hair. The second of these procedures was carried out by reshaving the axilla after the elapse of a definite period of time. On the basis of results from tests described below, an interval of 42 days was allowed to elapse between shavings. This period of time provided a mass of hair adequate for study, yet was brief enough to prevent introduction of errors because of loss of hairs or the growth of a succession of hairs in the same follicle. The weight of a sample is expressed in terms of the number of mg. grown per day. For example, the average daily rate of growth is one mg., if the sample of hair obtained after an interval of 42 days weighs 42 mg.

The amount of hair grown per day bears a significant relationship to the total quantity of hair that contributes to this growth. Accordingly, growth is expressed preferably in terms of the ratio of the average daily rate of growth (mg.) to weight (mg.) of the full-grown mass of hairs. Thus, the value is 0.01 in a man who grows one mg. of hair per day and whose full-grown mass of hair weighs 100 mg.

TABLE 1

Sex	*Age in years*								
	15–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90+
Males	12	59	43	36	36	30	27	10	—
Females	11	20	32	32	24	34	25	7	1

Methods employed in the extraction and colorimetric assay of urinary ketosteroids, along with an analysis of these data, have been described elsewhere (Hamilton and Hamilton, 1948). The methods and results obtained in bioassay of androgens are also presented in detail elsewhere (Hamilton, Hamilton, and Mestler, 1951). In brief, the urine was collected for 3 to 6 days. Extraction and separation followed the procedures employed by Dobriner and colleagues (1948). Colorimetric assay was carried out by the method devised by Zimmerman (1936) and modified by Holtorff and Koch (1940). Androgens were bioassayed as described by Hollander and associates (1941), but with the utilization of statistical principles of assay as defined by Bliss (1944).

Results

Axillary hair is a secondary sex character, as shown by the following facts: (1) this hair does not appear until the time of sexual maturation; (2) its development is nil, or practically nil, in most subjects who were castrated prior to puberty; and (3) maintenance of this secondary sexual character, even after it has attained full growth, is dependent upon continued stimulation by gonadal secretions, since these hairs tend to atrophy and may disappear entirely upon removal of the testes or ovaries and following the menopause in women.

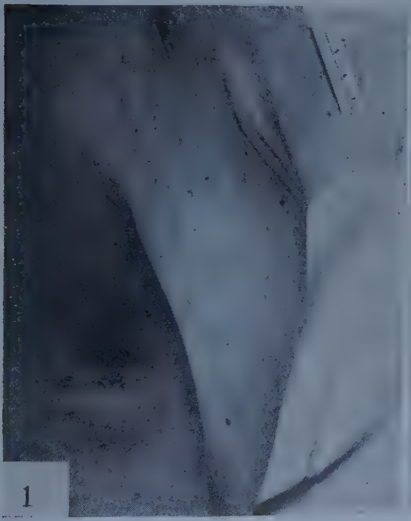
Scanty Growth of Axillary Hair in Eunuchs. In men who were castrated prior to sexual maturation, axillary hair is either absent or scanty (TABLE 2). FIGURE 1 illustrates the axilla of a man with less than 0.1 mg. of axillary hair who was 59 years of age at the time the picture was taken and had been orchiectomized at 12 years of age. FIGURE 2 shows a sparse distribution of fine hairs that is characteristic of the axilla of men castrated as adults.

TABLE 2

WEIGHT OF FULL-GROWN MASS OF HAIR IN RIGHT AXILLA OF EUNUCHS

Average age at		*No. of cases*	*Weight of hair in right axilla*		
Castration	*Examination*		*Average (mg.)*	*Range (mg.)*	*S. D. (mg.)*
8–12	33.75	12	6.72	0.1–25.0	9.67
14–16	27.50	10	8.46	0.1–32.2	9.40
17–19	28.96	13	22.34*	0.1–98.4	28.20
20+	41.63	35	20.30	0.1–138.9	30.20

*Among non-castrates, there is also a break in values at about 17 years of age.



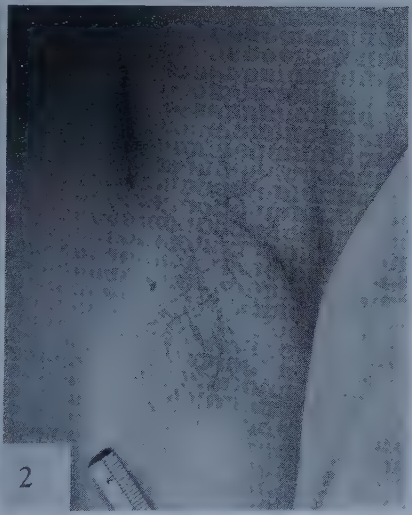


FIGURE 1. The scanty axillary hair (less than 0.1 mg.) of a 59 year-old eunuch who was orchiectomized at 12 years of age. As a result of castration prior to puberty, the secondary sex character of axillary hair failed to develop.

FIGURE 2. Illustration of the fine, sparsely distributed axillary hair (13.4 mg.) of a 47 year-old eunuch who was orchiectomized at 39 years of age. This secondary sex character became atrophic following castration of this sexually mature adult.

Although the hair tends to be more plentiful in men who were castrated as adults than in men who were castrated before they attained puberty, the quantity (TABLE 2) is considerably less than normal (FIGURE 4). The hair in FIGURE 2 weighed 13.4 mg. and belonged to a 47 year-old man who was orchiectomized at 39 years of age (average normal for age: 110 mg.).

In addition to reduction in the gross amount of axillary hair as a result of castration, the rate of growth of this hair is also diminished and its morphologic appearance altered. Hairs tend to lose kinkiness and pigmentation and are reduced in both length and diameter. These morphologic changes are reserved for discussion elsewhere in detail.

Data pertaining to weight of the full-grown mass of axillary hair in eunuchs is summarized in TABLE 2. The average weight of the hair in 12 men who were castrated before they were 13 years of age was only 6.7 mg. This contrasts with an average value of 126 mg. for normal men of the same age. A similarly small average weight, 8.5 mg., was found in 10 men who were castrated between 14 and 16 years of age. This is in contrast to an average of 146 mg. in normal men of the same age. The 48 men who were 17 or more years of age before orchiectomy retained a slightly larger mass of axillary hair, an average of 20.9 mg., but this amount is also much less than the average of 100 to 150 mg. for men of their general age-range (FIGURE 4). Only in four per cent of the cases did men who were castrated after they were sexually mature have values that approximated the average for normal men of their age. A similarity was observed between values for men and women who were gonadectomized after they had matured sexually.

Status of Axillary Hair in Relation to Titers of Urinary Ketosteroids and Androgens. In 43 men between 21 and 75 years of age, concomitant measurements were made of the weight of the full-grown mass of axillary hair and titers of urinary ketosteroids and androgens. In individual subjects, the correlation between the weight of axillary hairs and the titers of urinary steroids was by no means exact, whereas, in averages of groups of persons, a good relationship exists. This is illustrated for ketosteroids in FIGURE 3 for which the correlation coefficient was found to be + .493 ± .078.

Thus, average values for axillary hair could provide an index of urinary steroids in groups of subjects, but correlations in the individual person would not necessarily be in agreement, at least if only the weight of the full-grown mass of axillary hair is recorded. It is unfortunate that assays of urinary steroids were carried out at an early stage in this study, before the more precise analysis employing the ratio of hair growth was developed. Possibly the ratio of growth, which corrects for individual variance in growth of axillary hair, would result in better correlation of values for hair and steroids in single individuals as well as in groups of subjects.

Changes in Axillary Hair with Age. (1) *Weight of Full-Grown Mass of Axillary Hair.* Examination of 62 fetuses and full-term babies, whose status is described in another article in this monograph,* shows that axillary hairs do not grow to reach the surface of the skin prenatally. Thus, these hairs obviously do not exhibit the well-developed but transient stage of stimulation at the time of birth that occurs in secondary sex organs such as the prostate and external genitalia.

At the time of sexual maturation, axillary hairs emerge above the surface of the skin and undergo a sequence of morphologic changes similar to those described for pubic hair by Crampton (1908). In this sequence, the hair is first fine and silky, then pigmented, and, finally, kinky. Crampton showed that these three stages appeared concomitantly with successive advances in physiologic maturation. Greulich and his associates (1942), in their study of boys, have shown that these morphologic changes accompany sequential increases in titers of urinary androgens.

---

*P. 708.

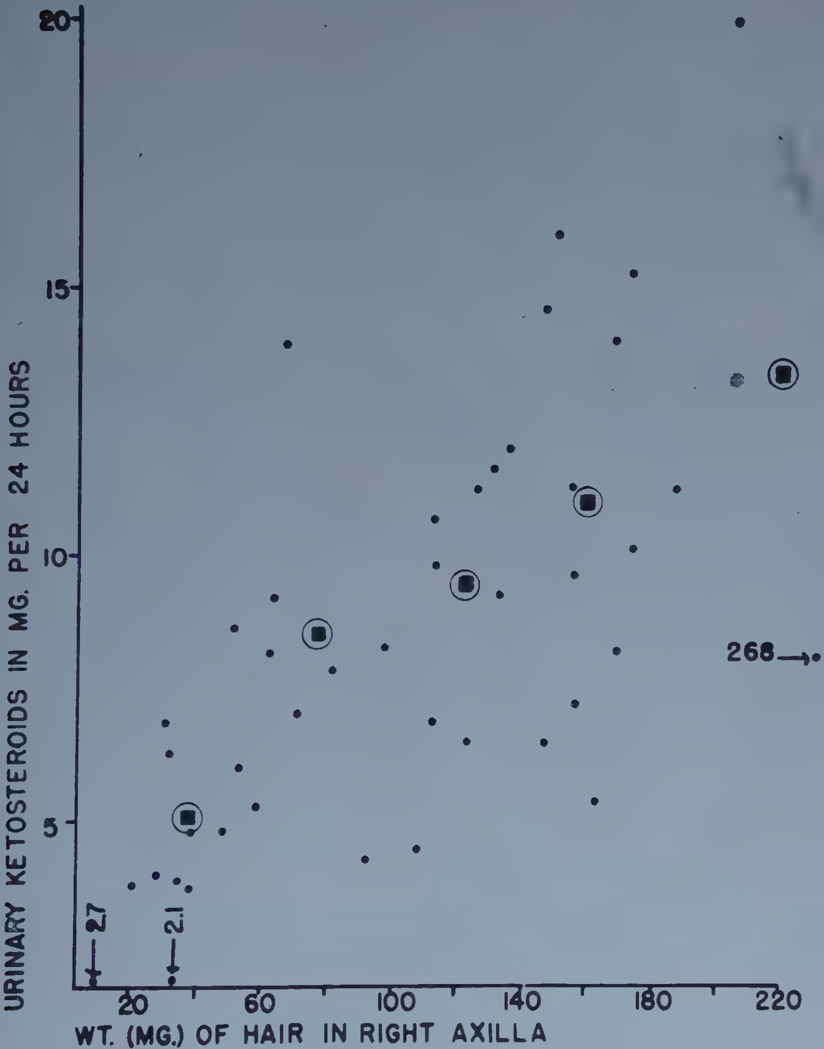


FIGURE 3. Comparison of the values for urinary ketosteroids and the weight of the full-grown mass of axillary hair. Dots represent data for each of the 43 normal men who were 21 to 75 years of age. Encircled squares are averages for individual values between 20 and 59 mg., 60 and 99 mg., 100 and 139 mg., 140 and 179 mg., and 180 mg. or more. In averages for groups of subjects, either in terms of weight of the full-grown mass of hairs or by decades of age (not shown in this graph), there is a correlation between titers of urinary ketosteroids and the weight of axillary hair. In individual subjects, the correlation is by no means exact.

(a) IN MALES (FIGURE 4). As shown in FIGURE 4, axillary hair begins to grow during the second decade of life and reaches a peak in the third decade. Thereafter, the average value per decade decreases materially and pro-

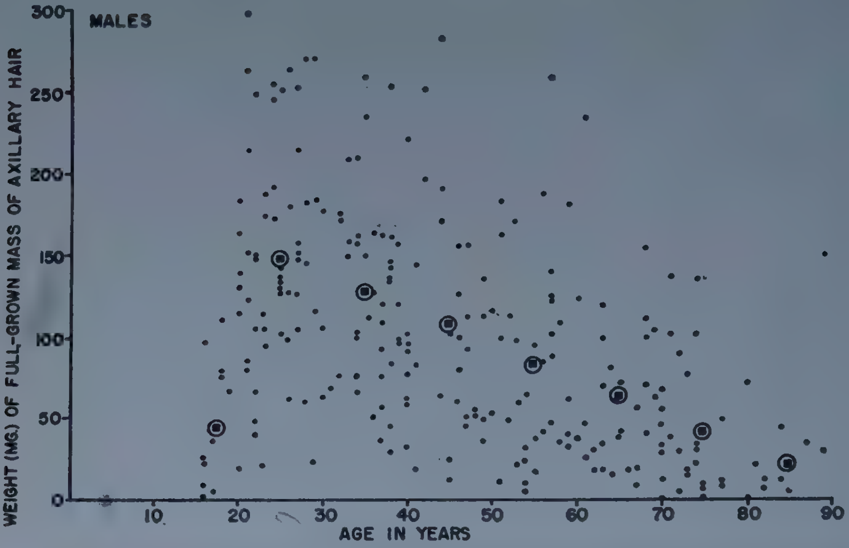


FIGURE 4. Graphic portrayal of the weight of the full-grown mass of axillary hair in males, from the second to the ninth decade of life. Dots represent values for individual subjects; encircled squares, the average value per decade.

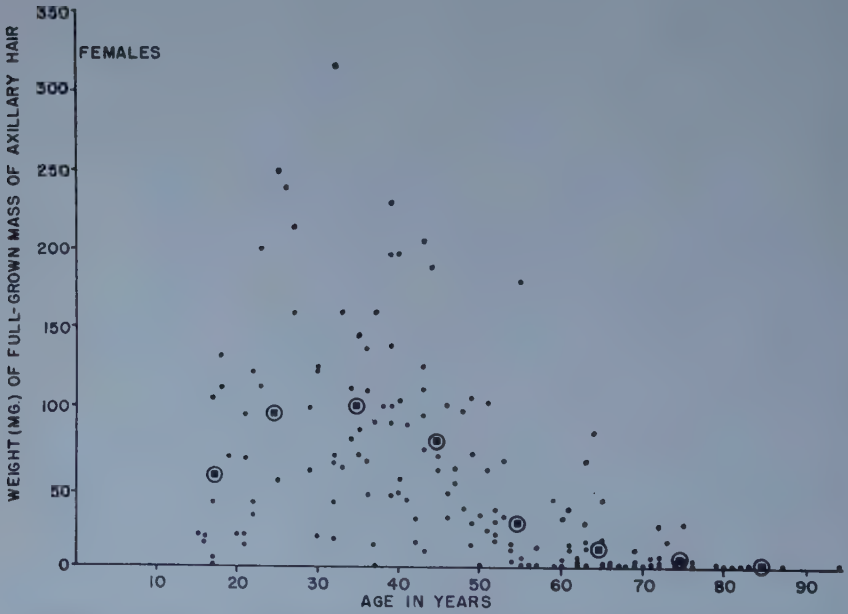
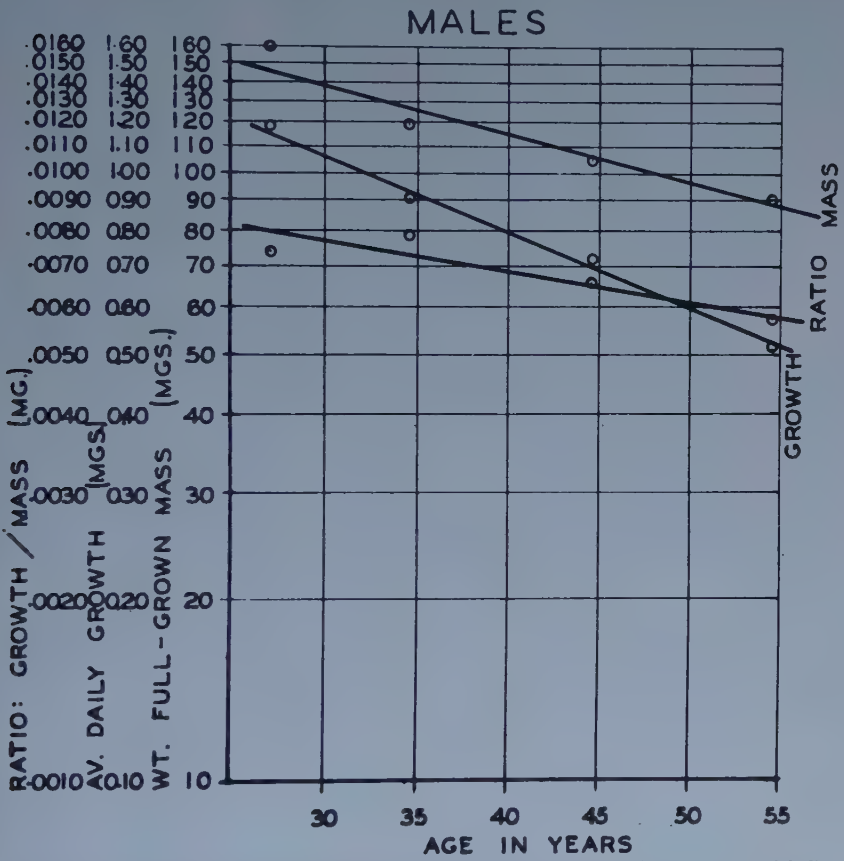


FIGURE 5. The weight of the full-grown mass of axillary hair in females from the second to the tenth decade of life. Dots represent values for individual subjects; encircled squares, the average value per decade.

gressively. The curve of decline with age is almost a straight line. In the men, the growth of axillary hair tends to continue to some extent even in old age.

(b) IN FEMALES (FIGURE 5). During the latter half of the second decade, the average weight of the full-grown mass of axillary hair is higher in females than in males. This is in accordance with the fact that girls mature at a chronologically earlier age than boys (Shuttleworth, 1938). As in males, the weight is maximal in the second and third decade of life, but the average values in these and in subsequent decades are not as high in females as in males. Thus, the average in the second decade is only about two-thirds as high in females as in males (96 *vs.* 146 mg.).



FIGURES 6 and 7. Summary, for males and females, 25 to 59 years of age, of the average rate of decline in the weight of the full-grown mass of axillary hair, in the rate of growth, and in the ratio of growth. The decrease with age in the weight of the full-grown mass of hair and in its rate of growth is more drastic in females than in males. For the ratio of growth, the slope of the curve of decline is similar in both sexes. (See FIGURE 7 on page 592.)

The decrease in the weight of axillary hair that occurs with aging appears earlier and is more drastic in females than in males. This reduction is greatest at the chronological age for the menopause and appears to be associated with the decline in ovarian secretions. At the chronological age when the menopause occurs, the weight of axillary hairs is less in women who no longer menstruate than in those who have continued to menstruate (unpublished data). A similar reduction in values has been observed in young women, following oöphorectomy.

Axillary hair practically ceases to grow in most old women. Only 13 per cent of 32 women, 66 or more years of age, had values greater than 6 mg. This is in contrast with the status in the 50 old men of this age-range, 90 per cent of whom had values greater than 6 mg.

Obviously, the stimulation to growth of these hairs that is provided in most normal old women does not continue to the extent observed in normal old men, and it may be assumed that ovarian secretions suffer a greater reduction with age than do testicular secretions. The variation in values for individual subjects is considerable, as was also the case in males (FIGURES 4 AND 5).

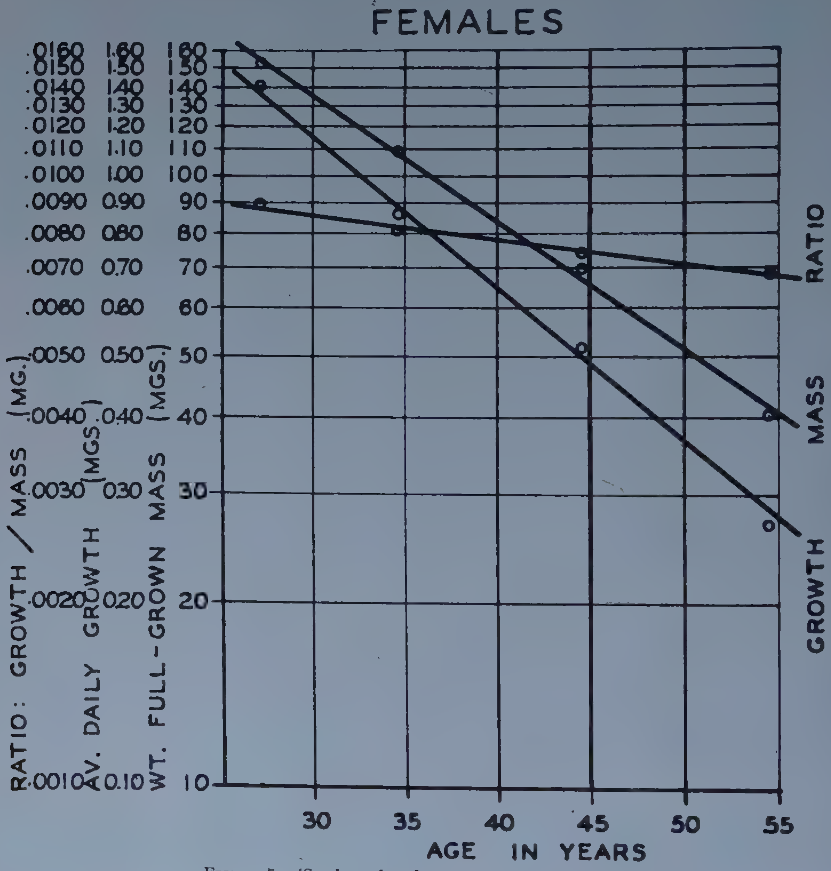


FIGURE 7. (See legend under FIGURE 6 on page 591.)

(2) *Rate of Growth.* The average daily rate of growth has been studied as yet only in subjects between the 25th and 59th year of life.

(a) IN MALES (FIGURE 6). The average rate followed the same type of curve as that for the weight of axillary hair in those same ranges of age. The reduction was progressive and drastic from peak values of the third decade.

(b) In Females (figure 7). The findings in females are similar to those in males, with the exception that the slope of the curve was greater and the decrease with age was more profound.

In both sexes, the variability in values for different individuals in the same range of age was as great as in measurements of the weight of the full-grown mass of hair in these subjects.

(3) *Relationship between Daily Rate of Growth and Weight of the Full-Grown Mass of Axillary Hair.* It seemed advisable to search for procedures which would reduce the great variability of values among different individuals, which is evident in both the weight of full-grown mass of axillary hair and in the amount grown per day. Based on the reasoning that the quantity of hair grown per day would depend upon the size of the parent mass of the axillary hair, data for these two items were plotted as shown in figure 8 for females. An excellent biological relationship is evident.

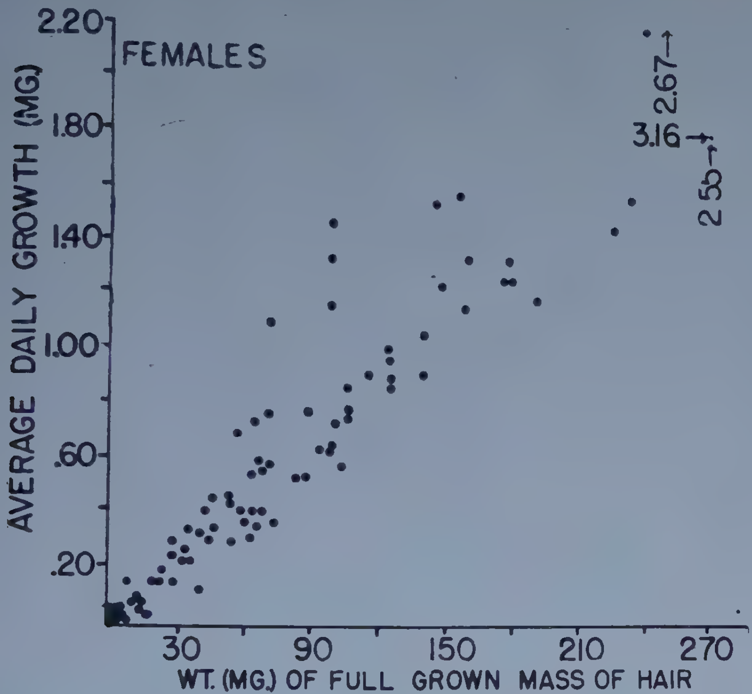


Figure 8. The amount of hair grown per day bears a close relationship to the quantity of the parent mass of hair.

(4) *Ratio of Growth.* Therefore, the method employed to measure the status of axillary hair in the following data is a ratio of growth which is the average rate of growth (mg. per day) divided by the weight (mg.) of full-grown mass of axillary hair. The variability in ratio of growth among different individuals is only about half of that observed with separate analyses of the weight of axillary hair and the daily rate of growth.

In figures 6 and 7, which summarize these three measures, it will be seen that the weight of axillary hair, and its daily rate of growth, decrease with age at a more rapid rate in females than in males, whereas the slope of the decline in the ratio of growth is approximately the same in the two sexes.

Factors which Influence the Growth of Axillary Hair. (1) Age, Sex, and Gonadal Secretions. The influence of these factors has been described above. Obviously, the reduction with age in growth of axillary hair occurs in association with diminishing titers of hormones (FIGURE 3), which may be responsible in good part for the decrease in growth. Available data do not make clear the extent to which the increase in growth of axillary hair during adolescence and the decrease in later years are the result of changes in the responsiveness of an individual to endocrine stimulation. Hooker (1942), Price (1944), and Caridroit and Garcet (1946) have indicated the responsiveness of rats and capons to exogenous androgen shifts at certain ages. Evidence to the contrary has been reported in capons by Gallagher and Koch (1935).

(2) Variations in the Same Individual from Time to Time. For 13 consecutive months, studies were made of 27 women and 26 men who were 25 to 59 years of age. Three other men and one woman were investigated for three to seven years. In most subjects, the variations from time to time amounted to as much as 50 per cent.

Averages of the 27 women and 26 men showed a tendency to the lower values in the winter and spring than at other times of the year. These averages smooth out a plethora of individual variations. Subjects who live in the same institution under identical regimes of life vary considerably with regard to the time of year when peak and low values were obtained. Not only do the variations from time to time differ among individuals but the values for the same individual differ at the same season in successive years.

Variations from time to time characterize the growth of the small amount of axillary hair present in some eunuchs, just as they characterize the growth of hair in normal subjects.

(3) Variation in Responsiveness of the Individual. The growth of axillary hair in response to androgenic stimulation appears to vary from time to time in the same person. This is shown by data such as those in FIGURE 9, which are from an orchiectomized man who received an unvarying dosage of testosterone propionate. Daily intramuscular injections of 30 mg. of testosterone propionate, dissolved in one cc. of peanut oil, were given at the same hour each day for several years. Use of this substantial amount of hormone minimizes the possibility that supplementary secretions by the adrenal cortex are responsible for the variation in growth of axillary hair.

While this orchiectomized man received an unvarying daily dosage of exogenous androgen, he exhibited variations from time to time in the growth of axillary hair (FIGURE 9) that are similar in magnitude and character to those exhibited by normal subjects.

(4) Genetic Factors. Values for axillary hair in subjects of Chinese or Japanese ancestry tend to be considerably lower than those for Caucasians of the same sex and age. The scale of values is so much lower that it is neces-

sary to establish a separate set of standards for members of the Mongoloid races. These subjects have a lesser growth of secondary male sex characters in general, an example of which, described elsewhere in this monograph,* is the limited degree of baldness in Chinese men. Another example is the less extensive growth of hair on the external ear in Chinese males than in Caucasian males (Hamilton, unpublished data). This character is a male secondary sex character that is not observed in normal women (Hamilton, 1946).

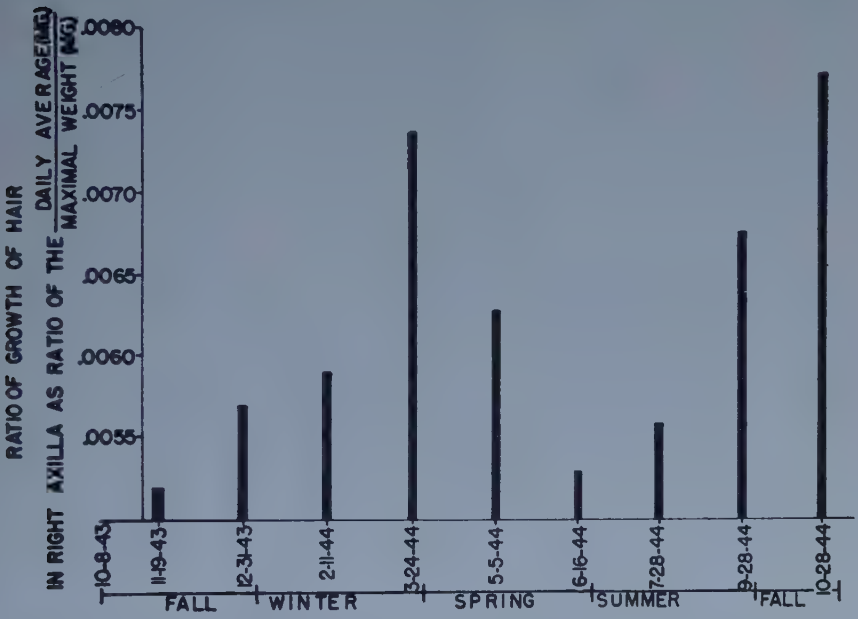


FIGURE 9. Variations from time to time in growth of axillary hair that were observed in an orchiectomized man while he received an unvarying dosage of exogenous hormone in the form of daily intramuscular injections of 30 mg. of testosterone propionate in one cc. of peanut oil. The nature and magnitude of the variations are similar to those in normal men and suggest that the responsiveness of the individual may fluctuate.

There is no evidence that testicular secretions are less in members of the Mongolian race than in Caucasians. Values for urinary ketosteroids are reported to be in the same range as in Caucasians (Chou and Wu, 1937). Thus, it must be presumed tentatively that the limited growth potential of axillary hairs in members of the Mongolian race may not rest on an endocrine basis. A genetic factor is suggested also by the fact that, among Caucasians, the members of some families tend to have relatively less growth of axillary hair than the general population. Unfortunately, measurements of titers of urinary steroids could not be made in these subjects.

At present, conclusions are not drawn as to whether a divergence from average values for the growth of axillary hair in Caucasians is necessarily associated with differences in the extent of development of secondary sex characters. Data presented elsewhere in this monograph* show, however, that weight of the full-grown mass of axillary hair is not significantly greater in men who are bald than in those who are not bald.

---

* P. 708.

Utility of Axillary Hair Values for Assessment of the Endocrine Status. After a description of the many factors which influence growth of this secondary sex character, it is perhaps well to show that this character can be profitably employed in evaluation of certain aspects of the endocrine status. FIGURE 10 shows values for the ratio of growth of axillary hair in a woman before, during, and after a pregnancy. After delivery, she failed to menstruate for several months and the growth of axillary hair was reduced almost to the vanishing point. Subsequently, this woman began to menstruate again and growth of axillary hair returned to its previous rate.

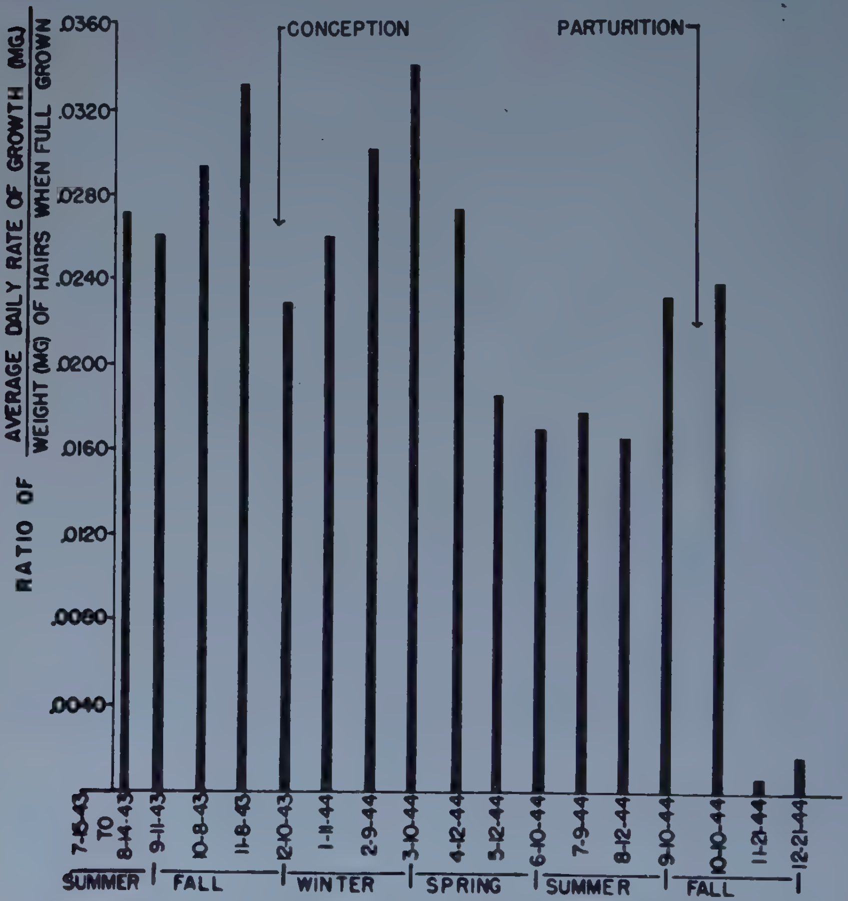


FIGURE 10. How the growth of axillary hair may be used to assess the endocrine status. For 6 months after parturition this woman failed to menstruate and the growth of axillary hair almost ceased.

Growth of axillary hairs can be studied with the use of simple procedures. Such studies can be carried out for either long or short periods of time.

Tests at intervals of 28 to 98 days between shavings gave comparable results (FIGURE 11), with the possible exception of one subject, whose hair grew at an exceptionally rapid rate.

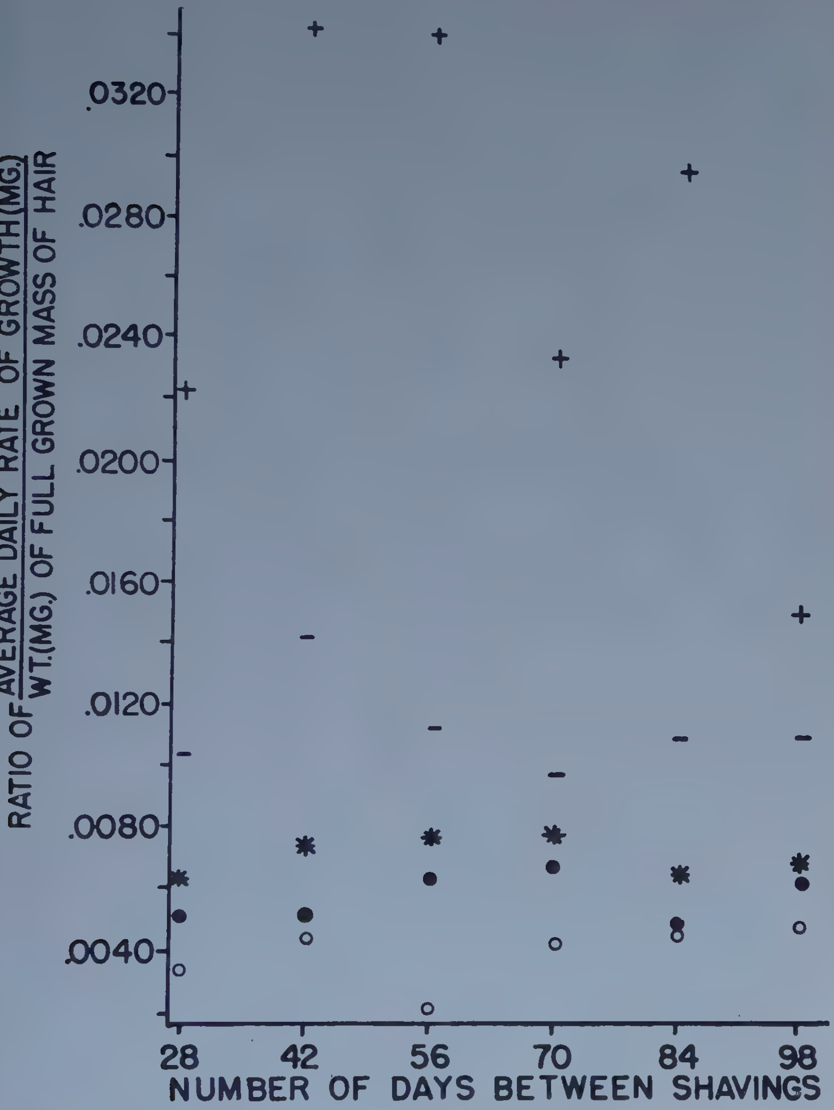


FIGURE 11. The intervals under test, namely 28 to 98 days, can be employed in study of the growth rate of axillary hair. In subjects whose hair grows at an exceptionally rapid rate, 98 days may possibly be too long.

Summary and Conclusions

Axillary hair is a secondary sex character, as shown by its absence until the time of sexual maturation; its failure to grow in men who were castrated prior to puberty; and by the atrophy of this character following castration of adult men or at the time of oöphorectomy or the menopause in women.

This character can be measured quantitatively by simple procedures and studied with ease for long or short periods of time.

These quantitative measurements can be used to assess certain aspects of the endocrine status. Values for axillary hair parallel those for urinary ketosteroids and androgens, if averages are made of groups of individuals. The parallel is by no means exact in data for individual subjects, and it remains to be shown which of these methods will provide the most valuable information with regard to each of various problems in the solution of which these methods might be of aid. In the light of the data available, quantitative measurement of axillary hair might well supplement, and to some extent replace, measurements of titers of urinary ketosteroids and sex hormones.

Data from the study of 448 subjects, between 15 and 92 years of age, may be used for at least preliminary standards in assessment of the growth of axillary hair in males and females.

Factors which influence growth of axillary hair are: inheritance; age; sex; gonadal secretions; responsiveness of the individual; and variations from time to time, some of which may reflect seasonal fluctuations.

Growth of this secondary sex character in normal people results chiefly from gonadal secretions. Very low values were found in the great majority of a series of 70 eunuchs, which would indicate that, ordinarily, the secretions of the adrenal cortex and the steroids produced by organs other than the testes, do not exert a significant effect upon growth of this character.

Bibliography

Bliss, C. I. 1944. Relative potency as applied to the assay of penicillin. Science **100:** 577–578.

Caridroit, F. & S. Garcet. 1946. Réponse a l'hormone male de la crête de vieux chapon. Compt. rend. Soc. de biol. **140:** 81–83.

Chou, C. & H. Wu. 1937. Contents of sex hormones in normal and pathological urine. Chinese J. Physiol. **11:** 429–435.

Crampton, W. C. 1908. Physiological age—a fundamental principle. Am. Physical Educ. Rev. **13:** 141–154, 214–227, 268–283, 345–358.

Dobriner, K., S. Lieberman, & C. Rhoads. 1948. Studies in steroid metabolism. I. Methods for isolation and quantitative estimation of neutral steroids present in human urine. J. Biol. Chem. **172:** 241–262.

Gallagher, T. & F. Koch. 1935. The quantitative assay for the testicular hormone by the comb-growth reaction. Second Communication. J. Pharmacol. & Exper. Therap. **55:** 97–126.

Greulich, W. W. 1942. Somatic studies. Somatic and endocrine studies of pubertal and adolescent boys, by Greulich, Dorfman, and Catchpole. Monogr. Soc. Res. Child. Devel. **7** (3): 85.

Hamilton, J. B. 1946. A secondary sex character that develops in men but not in women upon ageing of an organ present in both sexes. Anat. Rec. **94** (suppl.): 22–23.

Hamilton, H. B. & J. B. Hamilton. 1948. Aging in apparently normal men. I. Urinary titers of ketosteroids and of alpha-hydroxy and beta-hydroxy ketosteroids. J. Clin. Endocrin. **8:** 433–452.

Hamilton, J. B., H. B. Hamilton, & G. Mestler. Unpublished data.

Hollander, F., R. T. Frank, & E. Klempner. 1941. A dosage response equation for androgen assay by the chick comb method. Proc. Soc. Exp. Biol. & Med. **46:** 1–5.

Holtorff, A. & F. C. Koch. 1940. The colorimetric estimation of 17-ketosteroids and their application to urine extracts. J. Biol. Chem. **135** 377–392.

HOOKER, C. W. 1942. Pubertal increase in responsiveness to androgen in the male rat. Endocrinol **30**: 77-84.

KINSELL, W., E. REIFENSTEIN, D. BRYANT, & F. ALBRIGHT. 1946. The quantitation of axillary hair growth as an index of endocrine function. Assoc. for Study of Internal Secretions, Abstracts, 28th Annual Meeting, 16.

KOCH, W. 1921. Über die Russisch-Rumänische Kastratenseket der Skopzen. 39 pp. Fischer. Jena.

PRICE, D. 1944. The development of reactivity in the accessory reproductive organs of castrated and spayed rats injected with testosterone propionate. Physiol. Zool. **4**: 377–390.

PRIESEL, R. & R. WAGNER. 1929–1931. Gesetzmässigkeiten im Auftreten der extragenitalen sekundären Geschlechtsmerkmale bei Mädchen. Ztschr. f. d. ges. Anat. **15**: 333–352.

REYNOLDS, E. & J. WINES. 1948. Individual differences in physical changes associated with adolescence in girls. Am. J. Dis. Child. **75**: 329–350.

SHUTTLEWORTH, F. 1938. The adolescent period: A graphic and pictorial atlas. Monogr. Soc. Res. Child. Devel. **3** (3): 246.

ZIMMERMAN, W. 1936. Colorimetrische Bestimmung der Keimdrüsenhormone, Ztschr. f. physiol. Chem. **245**: 47–57.

THE ROLE OF THE PILIARY SYSTEM IN MAMMALS AND ITS RELATION TO THE THERMAL ENVIRONMENT

By L. P. Herrington
John B. Pierce Foundation, and Department of Public Health, Yale University, New Haven, Connecticut

If the evolutionary task of developing thermal properties in a class of animals with a "standardized" deep body temperature had been presented *a priori* to a group of thermal engineers with modern knowledge, they might have arrived at the following conclusions, which Brody[1] lists in connection with the temperature of homoiotherms. (1) Assuming constancy of body temperature, there must be an equality between heat loss and heat production. (2) Assuming equality of other conditions, heat loss is proportional to free surface. (3) Heat production is also proportional to oxygen consumption. (4) Since heat loss and heat production are proportional to free surface, and since surfaces vary with the squares of homologous sides, it follows that heat production and loss are proportional to the square of the corresponding dimensions of the animals under consideration.

These statements are a logical recognition of the fact that warm masses of greatly different size can be maintained at a fixed internal temperature in a cool environment at an energy cost proportional to exposed surface or to a power of the total weight less than one. This is essentially the so-called "Surface Law" of animal heat production and heat disposal.

Our hypothetical engineers would also have reached some surprising conclusions in relation to the variation in metabolic power required in equal tissue weights of very small and very large warm-blooded animals. This factor of heat input varies from approximately 8 cal. per kilogram for the elephant to 200 for the mouse. Such a range in tissue metabolic power is a necessary condition of a standard internal temperature in animals of greatly different size exposed to a common environmental temperature. The environmental temperature is not constant, however, and these animals would be expected to survive, despite variations of at least ±30°C around a mean terrestrial surface temperature of 15°C. At this point, our engineers would, no doubt, have thought of the heat insulative properties of the piliary system, and concluded that the problem could be solved by giving each animal a seasonally variable coat of hair, fur, feathers, or fleece, whose thermal insulation value could also be varied by piloerection to accommodate diurnal and other short term temperature changes. At this point, the question might be asked, "What are the quantitative limitations to peripheral insulation?"

As Kleiber[2] has shown in an interesting calculation, the use of a protective hair coat, which is dimensionally practical with regard to requirements of locomotion, is feasible only under the condition in which the metabolic power of animal tissue increases with decreasing animal weight. If this basic metabolic adjustment did not exist, a 60 gram animal, with metabolism comparable on a weight basis with a 600 kg. animal, would require a hairy coat some 8 inches thick in order to survive his temperate zone habitat temperatures.

From these illustrations, we see that the piliary system is, from one point of view, a variable thermal resistance which accommodates a basic tissue heat production conditioned by animal size to a seasonal factor. From a second point of view, the average insulative value of animal coats, without respect to season, can be seen as a factor which may increase or decrease the tissue heat production level which would otherwise be required by a given surface area and body mass. In both roles, peripheral insulation acts to extend the environmental range of a basic temperature regulation capacity in which chemical regulation is a very conspicuous feature.

The Physical Features of Piliary Insulation. How efficient is the outer coat of the animal as a thermal insulator? Although excellent studies are available on the physical and thermal characteristics of man-made derivatives of animal fur and fleece, we have very little convincing data determined by direct studies on normal and shorn animals under controlled calorimetric conditions. From physical studies that are available, we can state with confidence that the thermal protective value is primarily a question of thickness rather than weight. To put it dramatically, tissue thin ribbons of steel wool in random arrangement, entrapping thickness of air comparable to those entrapped by fleece, are not greatly different in insulative character from natural fleece. Steel wool, by reason of the matting characteristics and lack of hydroscopic properties, would not, of course, make a satisfactory animal coat. The illustration, however, emphasizes the well-established fact that it is the capacity of fleece, feathers, and wool to stabilize a thickness of air that is the important thermal property, and not the intrinsic thermal capacity or thermal conductivity of the fiber itself.[3]

In fact, small layers of motionless air in successive laminations provide the ideal outer insulation. The down of the eider duck is, perhaps, one of the best biological approximations to the physical ideal. In the same sense, the mechanism of variable piloerection, no doubt controlled by sensory cues in the animal, is a mechanism which effectively alters the insulation factor of the hair coat through physical variation of the degree of effective air layer lamination.

As insulating layers are built up on the animal body, the protective efficiency of successive thickness decreases in accord with the law of diminishing returns. There is a limit, therefore, to practical insulation values that may be attained by piliary development. The body segments of animals usually approximate cylinders. Physical studies have shown that the efficient thickness of insulation is reached more rapidly on small cylinders than on larger cylinders.[3] Thus, in human exposure, addition of insulation much beyond $\frac{1}{4}''$ in thickness is of small value on the fingers. In contrast, the trunk would benefit materially from 5 to 6 inches of insulation, if such thickness were otherwise practical. A survey of the furred and fleeced mammals demonstrates that this technical point in optimum piliary thickness is apparently appreciated, or shall we say assayed, by the biological mechanisms controlling piliary development.

To answer our original question as to the thermal protection afforded by animal coats, we can offer values based on the theory of the dead air space in lieu of other more direct data. An average value for a fine fibered fleece specimen may be expected to have a thermal resistance of the order of .72°C per kg.cal./M^2/hr./2.5 cm., or 1 inch of fleece depth. This means literally that, for each kilocalorie of heat production per square meter per hour, the skin surface of the animal will be .7°C warmer than its outer fleece surface. Relating this to the figure of 1000 kg.cal./M^2/24 hours, frequently cited as a representative value for the basal heat production of the entire homoiothermic group, we find the following relations. A resting warm-blooded animal with a fleece or fur coat of 2.5 cm. thickness must pass approximately 41 cal./M^2/hr. The product of the thermal resistance of the fleece and the thermal head is 41 × .72, or a 29.5°C difference between true skin and outer fleece surface. A very large number of warm-blooded animals, when on a plane of nutrition comparable to the basal value of 1000 kg.cal./M^2/24 hours noted above, are in a state of neutral thermoregulation at air temperatures of 30°C when shorn, or, in the case of man, without clothes.

We may reasonably conclude, therefore, that addition of 2.5 cm. of fine fleece or fur permits environmental temperature to be dropped approximately to 0°C without altering body or skin temperature significantly from the values that obtain in the thermally unstressed and unfleeced animal at 30°C. Higher planes of nutrition will materially lower the point of thermal neutrality, and hence extend the point of effective protection values to lower points on the thermometer scale.

From a knowledge of habitat temperatures and the range of thickness in animal coats, it is easily seen that, with reserves of chemical regulation and the possibility of higher planes of nutrition, the thermal adjustment of polar animals to temperatures of $-30°$ and $-40°C$ is well within practical limits. It is also obvious that rapid alteration of fur and fleece character under endocrine control is quite necessary, if heat stress is to be avoided during the moderate season.

As a theoretical value, it is interesting to estimate the degree to which the piliary insulation approximates the limiting value afforded by an equal thickness of absolutely still air. This has been done.[4] If the 2.5 cm. fleece above had been an ideal insulator, the effective gradient set-up between skin and outer fleece surface would have increased from 30°C to 51°C. In short, the biological version of insulation is about 57 per cent of the theoretical maximum for the thickness involved.

The Relative Importance of the Thermal Protection of the Piliary System in Animals of Different Sizes. In the smaller ranges of animal size, the practical role of the piliary system in relieving chemical regulation against cold stress is quantitatively much less impressive than in the larger animals. It is well established that variations in habitat temperature of the order of 20° to 25°C can reduce the average life span of such animals as the rat and mouse by a factor of 2 or 3, the longer life span being experienced in the warmer environment. This fact is usually related to the higher metabolism under cold conditions, on the assumption that the integral of metabolic power for a given species has a typical value which may be reached at different rates, dependent upon the speed of living. The inability of a practical degree of coat development to compensate fully for even moderate temperature changes in such animals as the rat is well illustrated by the metabolic response of these small animals to cold stress.

In FIGURE 1, the effect of a 20° variation in environmental temperature for the albino rat is illustrated. Animals in this test were acclimatized to a temperature of 24°C and the ordinate values indicate the metabolic response to 7 hours exposure under basal conditions to air temperatures between 13° and 35°C. Minimal metabolism, the so-called critical temperature, or thermal neutrality point, is observed for these rats at 28° to 29°C. Despite acclimatization to a room temperature significantly below this point, the piliary insulation of this species is not sufficiently variable through piloerection to obviate the need for chemical regulation. In fact, the total heat production is increased by approximately 100 per cent when the animals are exposed to 12° to 13°C, temperatures which are very common in the seasonal cycle of temperature zone.

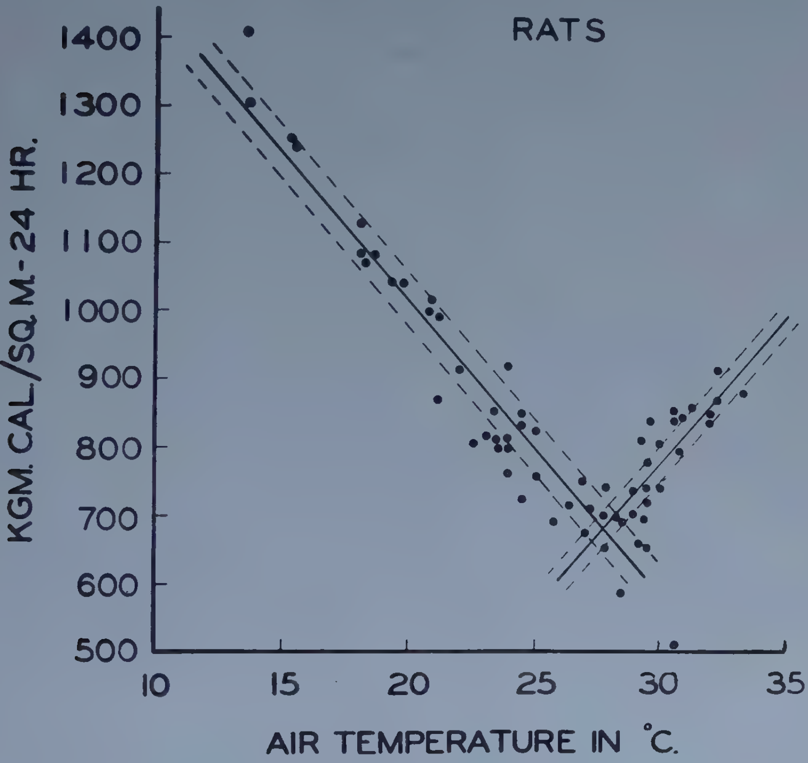


FIGURE 1. Standard metabolism of albino rats at various temperatures. Each point represents the mean of 10 animals.

It would be expected, however, that acclimatization to 12°C would result in piliary growth that would considerably moderate the chemical regulation required at this temperature, as shown in FIGURE 1. In other tests from our laboratory,[5] a physical value has been determined for the insulative change in the albino rat's coat when comparable groups are raised at 18° and 31°C. The results are interesting primarily in comparing the relative dependence of this animal on chemical and piliary insulation over a moderate range. Although a shift in temperature from 31° to 18°C increased chemical regulation by 50 per cent, the increase in insulation value of the coat was approximately 20 per cent. In other animal species, and particularly in animals living in their natural habitat, seasonal changes in piliary protection by a very much larger order would be expected.

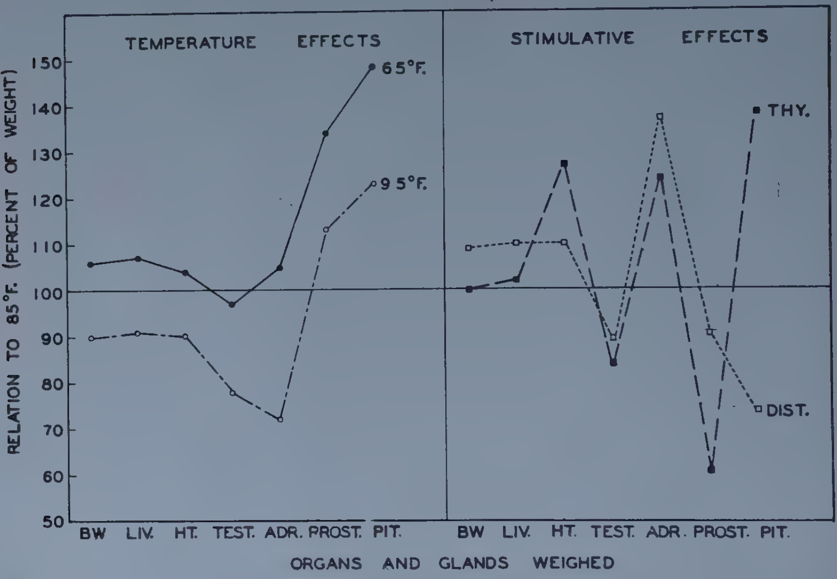


FIGURE 2. Average organ and gland weights (related to body weight) of albino rats, living at 65°F and 95°F (210 days), referred to a base line (100 per cent) representing similar data for a control group living at 83°F.

Seasonal Adaptations to Temperature Change. In view of other papers in this monograph, an extended consideration of the metabolic mechanisms which are concerned in the seasonal adaptations of the piliary system is not required. It is obvious from many sources that the piliary response is one feature of a complex metabolic change in which endocrine factors play a conspicuous role. In this connection, it may be of casual interest to report the pattern effect of endocrine weights of different environmental temperatures. This factor was studied in the same colony of albino rats on which the above metabolic and piliary insulation data was secured. In FIGURE 2, the mean organ weight relative to body weight[6] has been compared in 3 groups of 20 rats each. The horizontal line, at ordinate 100, represents the reference weights of organs in 20 rats living at a temperature of 83°F for 210 days. The pattern lines marked 65°F and 95°F represent the effect of these temperatures in producing deviations in organ weight as compared with the 83°F group. The right section of FIGURE 2 may be disregarded. The most interesting item in this gross comparison is the remarkably similar pattern effect produced by temperatures low enough to stimulate chemical regulation or temperatures high enough to increase heat production through body temperature elevation. The adrenal weights are the only exception to this pattern similarity, which exists irrespective of the depressing or stimulative effect relative to the normal temperature group at 83°F.

If we consider these data in conjunction with the studies of Landauer and others on the metabolic characteristics of genetic variants without feathers or hair, it is reasonably certain that the degree and character of piliary insulation probably has an important reciprocal effect on the metabolic and endocrine systems through piliary capacity to alter the apparent temperature of the environment.

Estimates of Peripheral Insulation in Relation to the Determination of Favorable Habitat Temperatures for Domesticated Animals. A useful fact derived from the study of the thermal insulative value of animal coats is the possibility of predicting for domestic animals the habitat temperatures which will correspond to thermal neutrality with various planes of nutrition. Obviously, the data derived from determinations of the temperature of minimal metabolism under basal conditions does specify directly the environmental temperature points which are associated with similar heat regulation status under conditions of high nutrition and the normal habit. By application of the concepts of partitional calorimetry,[7] it is possible to find convenient answers to this problem, leaving to animal nutritionists the solution of the question as to which phase of temperature regulation is most favorable for animal health or most profitable from the standpoint of animal economics.

TABLE 1

SUMMARY OF INSULATION RESISTANCES* IN PATH OF HEAT FLOW FROM INTERNAL BODY REGIONS TO THE ENVIRONMENT FOR VARIOUS FARM ANIMALS

	Peripheral tissues	*Hair or feathers*	*Surface to air*
Cow	0.066	0.112	0.132
Sheep	0.066	0.290	0.132
Goat	0.066	0.208	0.132
Pig	0.100	0.169	0.132
Hen	0.033	0.401	0.132

*The numerical values given are in terms of °C/kg. cal./hr./sq. meter-body surface.

In order to establish temperature points which are comparable, despite variations in level of heat production and peripheral insulation, one must know the experimental constants which relate heat loss from the surface of animal bodies to temperature differentials between the animal and the environment. In addition, information is required on the physical heat conductivity of the peripheral tissues and hair or feather coat. When these factors are known, it is possible by the calculation methods of partitional calorimetry to estimate habitat temperatures which will be associated with minimal temperature regulation stress for any plane of nutrition.

In TABLE 1, experimental values of the insulation of hair or feathers and a mean experimental value for the heat loss coefficient of animal surfaces have been assembled with estimated values for peripheral tissue insulation. The coefficients represent a total resistance as determined from measurements of temperature gradients between the successive surfaces concerned.

Details of the necessary calculations may be understood from the references on partitional calorimetry. It will be sufficient for the interest of this group to note the wide range of coat insulations in the cow, sheep, goat, pig, and hen, differing by a factor of nearly 4 between cow and hen. The insulation values available do not include a seasonal range, hence those cited may be considered as first approximations. When such values are applied to the metabolic data for these various species, an environmental temperature may be derived which represents, for a given plane of nutrition, the point of minimal temperature regulation stress.

TABLE 2

CALCULATED VALUES FOR THE TEMPERATURE OF MINIMAL HEAT REGULATION STRESS FOR GIVEN PLANES OF NUTRITION AND AVERAGE PILIARY INSULATION

Animal	*Cow*	*Swine*	*Sheep*	*Hen*
Av. heat production, basal units.*	3.0	1.66	1.66	1.66
Typical weight, lbs.	1000	300	85	5
Temp. of minimal heat regulation stress, °F.	45	60	53	53
Surface area, M^2	6	3.7	1.17	0.17
Total sensible heat output at av. heat production	570†	195	61.5	5.9
Total water vapor	136†	46.8	14.8	2.9
	237§	81.4	25.8	5.0

*Under average feed and farm conditions. One basal unit is taken as 1000 kg. cal./m^2/24 hrs.
†Kg. cal. per animal per hour.
‡Kg. cal. per animal per hour, evaporation.
§Grams of H_2O vapor per animal per hour.

Summary data of this type are presented in TABLE 2. In the third line, the calculated temperatures of minimal temperature regulation are given for the plane of nutrition noted in the first line. It is a surprise to many who see these data to find a temperature of 45°F, which is decidedly cold by human standards, given as a neutral point for the cow. Inspection of the first line will show, however, that a high plane of nutrition, three times the basal value, has been specified, and such values are common in cows under milking regime. Of the three remaining species, the data for the hen is in accord with experience. The values calculated for swine and sheep are accurate with respect to the limited skin temperature data available for these species, but the degree to which the reported experiments represent average conditions of coat and fat development are not exactly known.

Summary

The essential conditions which permit the maintenance of a constant deep body temperature in homoiotherms have been described. Over the wide range of animal sizes, adjustment in the metabolic power of animal tissue is the most significant factor in permitting a regulated temperature. Within a given species, piliary development assumes a major role in conjunction with chemical regulation in providing thermal protection. The relative contribution of piliary insulation increases as the absolute size of the animal increases, hence is of nominal value to the rat, increasing to major proportions in such animals as the sheep.

By appropriate combinations of physical and biological data, the effect of peripheral hair or feather insulation, on shifts of the temperature of minimal heat stress with plane of nutrition, may be calculated. Additional physical information on temperature gradients in the peripheral region of animals, under different conditions of coat development, is highly desirable. Such data would contribute substantially to a number of problems in the hygiene of the domesticated animal. From the standpoint of general biology, such data would also permit the construction of a standard biological scale of temperature. In the absence of a scale which corrects for the thermal effect of piliary development, no inter-species comparisons of endocrine or metabolic reactions can be said to be fully controlled with respect to the important metabolic stimuli associated with habitat temperature.

Bibliography

1. Brody, S. 1945. Bioenergetics and Growth. 1023 pp. Reinhold. New York.
2. Kleiber, M. 1932. Hilgardia **6:** 315.
3. Siple, P. 1949. Physiology of Heat Regulation (12). Edited by L. H. Newburgh. W. B. Saunders. Philadelphia.
4. Siple, P. 1949. *ibid:* 445.
5. Herrington, L. P. 1940. The heat regulation of small laboratory animals at various environmental temperatures. Am. J. Physiol. **129:** 123–139.
6. Herrington, L. P. & J. H. Nelbach. 1942. Relation of gland weights to growth and aging processes in rats exposed to certain environmental conditions. Endocrin. **30:** 375–386.
7. Winslow, C., E. A. Winslow, & L. P. Herrington. 1949. Temperature and Human Life. Princeton University Press. Princeton.

CHEMICAL CLASSIFICATION OF KERATINS

By Richard J. Block
Department of Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, New York, N. Y.

It is becoming increasingly apparent that the total proteins of an homologous series of tissues have a remarkably constant amino acid composition. TABLE 1 shows the amino acid patterns of washed and fat-extracted muscle proteins from various classes of vertebrates: mammals, birds, amphibia, fish, and crustacea. It will be seen that, within the experimental error of the method, there is no difference in amino acid pattern over practically the entire range of animals. Thus, the total proteins of muscle, from whatever animal they may be derived, have the same amino acid pattern. An analogous situation appears to hold in the case of milk from the few species that have been investigated (TABLE 2). Of the twelve amino acids studied, only cystine appears to show a significant deviation. TABLE 3 shows that even the amino acid patterns of entire animals and birds are also very similar. The collagens and elastins, obtained from mammals, marsupials, birds, amphibia, and fish, have been analyzed for eighteen amino acids under strictly comparable conditions. Of all the collagens and elastins studied, only fish skin collagen showed a significant deviation in three of the eighteen amino acids (methionine, serine, and threonine), while turtle collagen deviated from the majority only in threonine. Other tissue proteins, such as liver, stroma, hemoglobins, *etc.*, show remarkably similar amino acid patterns, and, if any marked differences are encountered, they are usually confined to the sulfur amino acids and possibly to the hydroxyamino acids.

A group of tissue proteins of ectodermal origin such as hair, nails, horn, feathers, *etc.*, have been recognized for the past seventy-five years as having certain features in common. These proteins are resistant to digestion by pepsin and by trypsin and are insoluble in dilute acids, alkalies, water, and organic solvents. They are called keratins, from the Greek word "*kéras*," horn. Studies initiated about twenty years ago showed that keratins could be divided into two groups which had approximately the same physical properties but which differed in the molecular ratios of their basic amino acids, arginine, histidine, and lysine. The one group comprising horn, hair, *etc.* were called eukeratins and yielded, on acid hydrolysis, histidine, lysine, and arginine in a molecular ratio of approximately 1:4:12. The other group, which did not show this ratio of basic amino acids, were designated pseudo-keratins. Only the eukeratins will be discussed in this paper.

TABLE 4 gives the amino acid patterns of four typical hair proteins. A calculation of the molecular ratios of the amino acids of these four types of hair, as well as many others not given in this table, shows that, although the majority of amino acids fall into approximately the same pattern, samples of hair from different species show marked variations in the molecular ratios in one or more of the amino acids investigated, except for the basic and acidic amino acids.

TABLE 1
SOME AMINO ACIDS IN MUSCLE PROTEINS

	Gm. per 16 gm. of N				
	Beef	*Chicken*	*Frog*	*Fish*	*Shrimp*
Arginine	6.9	6.9	6.6	6.4	6.6
Histidine	2.3	2.3	2.1	2.3	1.8
Lysine	8.1	8.4	8.0	9.0	8.3
Tyrosine	4.3	4.2	4.7	4.4	4.7
Tryptophan	1.4	1.3	1.4	1.4	1.2
Phenylalanine	4.9	3.9	4.7	4.5	4.8
Cystine	0.9	0.8	0.9	0.7	1.0
Methionine	3.1	3.4	3.2	3.8	3.4
Serine	5.4	4.7	6.3	4.0	4.0
Threonine	4.6	4.7	4.9	4.2	4.0
Leucine	9.2			7.3	
Isoleucine	4.8			5.0	
Valine	6.0			5.6	

TABLE 2
SOME AMINO ACIDS IN MILK PROTEINS

	Gm. per 16 gm. of N		
	Human	*Cow*	*Ewe*
Arginine	3.7	3.4	3.2
Histidine	2.7	2.6	2.7
Lysine	7.2	8.3	8.4
Tyrosine	5.4	6.4	
Tryptophan	1.5	1.4	
Phenylalanine	5.3	4.7	4.7
Cystine	1.6	0.7	
Methionine	2.2	2.7	2.5
Threonine	4.5	4.5	4.3
Leucine	9.8	9.0	9.6
Isoleucine	7.5	5.7	5.5
Valine	8.8	7.0	7.4

TABLE 3
SOME AMINO ACIDS IN WHOLE ANIMALS

	Gm. per 16 gm. of N	
	Rat	*Chicken*
Arginine	5.9	5.8
Histidine	1.8	1.5
Lysine	6.1	6.3
Phenylalanine	3.4	4.2
Methionine	2.0	1.6
Threonine	3.7	3.7
Leucine	6.4	7.2
Isoleucine	3.8	4.2
Valine	4.5	5.4
Glutamic acid	13.9	11.6
Aspartic acid	8.1	8.2
Glycine	10.0	11.0

TABLES 5 and 6 give the composition of three typical horn keratins of feathers and of two species of quills. Here again, molecular ratios of the basic amino acids and of aspartic acid are relatively constant, although there appear to be differences in the amounts of glutamic acid which may exceed the experimental errors of the analytical method.

TABLE 4
AMINO ACIDS IN HAIR KERATINS

	Gm. per 16 gm. of N			
	Human hair	*Chimpanzee hair*	*Swine hair*	*Lamb's wool*
Arginine	9.6	8.1	10.5	10.1
Histidine	0.9	0.6	1.1	0.9
Lysine	2.6	2.0	3.7	2.8
Tyrosine	3.1	3.3	3.4	5.5
Tryptophan	0.7	1.4		0.7
Phenylalanine	2.7		2.6	4.0
Cystine	16.0	15.5	13.9	13.6
Methionine	1.0		0.5	0.7
Serine	7.6			9.0
Threonine	7.2		6.1	6.5
Leucine			8.0	7.8
Isoleucine			4.5	4.2
Valine			5.7	5.1
Glutamic acid	14.8		17.2	15.4
Aspartic acid	8.0		7.7	7.1
Proline			9.3	8.0

TABLE 5
AMINO ACIDS IN HORN KERATINS

	Gm. per 16 gm. of N		
	Finger nails	*Cattle horn*	*Rhinoceros horn*
Arginine	10.0	8.6	8.3
Histidine	0.5	0.6	0.6
Lysine	2.8	2.4	2.7
Tyrosine		5.7	8.8
Tryptophan		0.7	1.7
Phenylalanine		3.3	5.1
Cystine	12.9	12.4	8.9
Methionine	1.0	0.5	1.5
Serine			7.0
Threonine		6.3	5.1
Leucine		8.5	9.3
Isoleucine		4.4	4.8
Valine		5.6	5.7
Glutamic acid	15.7	14.2	13.5
Aspartic acid	8.4	7.9	9.0
Proline			

TABLE 7 shows the amino acid composition of dental enamel keratin, as well as recapitulating the results on human hair and finger nails to facilitate comparison. It will be seen that, although the molecular ratios of the basic amino acids and methionine of dental enamel keratin are very similar to those of the other human keratins, its content of phenylalanine is quite different from that of hair, and its yield of cystine is significantly lower than that of any other eukeratin studied.

TABLE 6
AMINO ACIDS IN FEATHERS AND QUILLS

	Gm. per 16 gm. of N		
	Hen's feathers	*Porcupine quills*	*Echidna quills*
Arginine	8.0	7.7	7.2
Histidine	0.4	0.6	0.5
Lysine	1.4	2.6	1.9
Tyrosine	2.3	3.3	9.6
Tryptophan		0.9	2.7
Phenylalanine	5.5	3.6	7.2
Cystine	8.7	9.5	12.5
Methionine	0.5	0.8	1.1
Serine	10.2	6.2	6.0
Threonine	4.6	5.4	4.1
Leucine	8.5		
Isoleucine	6.4		
Valine	8.9		
Glutamic acid	10.4	17.8	9.3
Aspartic acid	7.5	8.8	6.9
Proline	9.4		

TABLE 7
AMINO ACIDS IN KERATINS OF HUMAN ORIGIN

	Gm. per 16 gm. of N		
	Hair	*Nails*	*Dental enamel*
Arginine	9.6	10.0	9.7
Histidine	0.9	0.5	0.9
Lysine	2.6	2.8	2.8
Tyrosine	3.1		
Tryptophan	0.7		
Phenylalanine	2.7		6.2
Cystine	16.0	12.9	0.6
Methionine	1.0	1.0	1.0
Serine	7.6		
Threonine	7.2		
Glutamic acid	14.8	15.7	
Aspartic acid	8.0	8.4	
Proline			

It was thought of interest to investigate the qualitative amino acid composition of five ectodermal structures from the same individual by means of paper chromatography. The results are summarized in TABLE 8. The sample of skin was the epithelial layer obtained after a mild attack of exfoliative dermatitis. The dandruff was obtained by combing the hair and then separating the scales from pieces of hair, *etc.*, by a fine screen. Although the dandruff weighed only a few mg. after extraction of the fat with lipoid solvents, sufficient material was obtained for this evaluation.

Hydroxyproline was not found on any of the chromatograms. The over-all picture one obtained from the chromatograms is that there is a similarity in the amino acid composition of these tissue proteins, except for proline and cystine, which are present in markedly smaller quantities in skin than in any of the other proteins. Dandruff occupies an intermediate position between skin and hair and nails with respect to cystine but is similar to the latter tissues in its content of proline. Skin may contain significantly larger quantities of phenylalanine, glycine, and methionine. It should be recalled that skin is probably not an eukeratin.

TABLE 8

APPROXIMATE MOLECULAR RATIOS OF AMINO ACIDS OF VARIOUS HUMAN KERATINS (TAKEN FROM ONE MAN)

Amino acid	*Hair*	*Finger nails*	*Toe nails*	*Skin epithelium*	*Dandruff*
Arginine	+[*]	13	+	+	10[†]
Histidine	+	10	+	+	17
Lysine	+	10	+	+	15
Tyrosine	+	11	12	+	10
Phenylalanine	10	10	12	17	11
Cystine	25	21	20	3	10
Methionine	+			+	+
Serine	+	10	10	+	10
Threonine	+	14	17	+	10
Leucines	+	11	11	+	10
Valine	15	14	13	11	10
Glycine	10	11	10	15	13
Alanine	+	14	15	+	10
Glutamic acid	+	10	10	+	11
Aspartic acid	+	10	11	+	12
Proline	25	22	23	10	24

[*] + indicates presence of amino acid with no indication as to concentration.

[†] The lowest molar quantity of each amino acid in any of the proteins has been assigned the value of 10, except in the case of cystine.

In conclusion, then, although it is recognized that the eukeratins discussed in this paper are heterogeneous proteins, it is believed that two major conclusions may be reached: first, in contrast to many other homologous tissue proteins, eukeratins show a wider range in their pattern of the majority of their amino acid components, and, secondly, they show a remarkable constancy in the molecular ratios of histidine to lysine to arginine.

Reference

BLOCK, R. J. & D. BOLLING. 1950. The Amino Acid Composition of Proteins and Foods. 2nd ed. C. C. Thomas. Springfield, Ill.

THE KERATINIZATION OF EPIDERMIS AND ITS DERIVATIVES, ESPECIALLY THE HAIR, AS SHOWN BY X-RAY DIFFRACTION AND HISTOCHEMICAL STUDIES

By A. Giroud and C. P. Leblond*

Department of Embryology, Medical School, University of Paris, Paris, France; Department of Anatomy, McGill University, Montreal, Canada

The production of keratin appears to be the chief metabolic achievement of the epidermis and its derivatives. The keratins, as we know them today, are proteins which contain variable amounts of sulfur and may be associated with lipids. Their chemical differences have led to a separation of the keratins by Unna into the so-called A and B types.[1] In turn, this has given rise to the concept of two corresponding processes of keratinization, *i.e.*, "soft" and "hard" keratinization.[2] In soft keratinization, the cells of the Malpighian layers become cornified after passing through a granular and a glassy stage, as may be seen in the epidermis, as well as in corns and callouses, the eponychium of nails, and the medulla of the hair.[2–4] An area of epidermis on the inner surface of the leg of the horse, the so-called horse burr, is thick enough to make possible a separation of the Malpighian from the cornified layer for chemical studies of soft keratinization. On the other hand, hard keratinization takes place by a progressive transformation of the Malpighian cells into cornified layers, through a transition or keratogenous zone without granular or glassy stages. Nails, horns, hooves, and claws, as well as the cortex and cuticle of the hair, are made up of hard keratin.[2–4] Thus, the hair combines the two types of keratinization, since it consists of a "soft" medulla and a "hard" cortex and cuticle.[2, 4]

Chemical Differences between Malpighian and Cornified Layers of the Epidermis and Its Derivatives. Since Malpighian and cornified layers are common to both types of keratinization, it is instructive to ask how these layers differ from, or are similar to, one another. The histological differences are the most obvious, since Malpighian cells show all the attributes of active function (well formed nuclei, mitochondria, *etc.*), while the cells of the cornified layers no longer show signs of vitality.

Histochemical studies also show the predominance of active metabolites in the Malpighian zone. Desoxyribonucleic acid[5, 6] and small amounts of ribonucleic acid[7] have been found in the cells of the Malpighian layers, but not in the cornified layers (see Dempsey's paper in this monograph). Another difference was brought to light by the demonstration of the presence of ascorbic acid in Malpighian layers of soft and hard keratin structures (0.08 to 0.14 mg./gm.), while less than 0.01 mg./gm. could be found in the cornified layers.[8, 9] Similarly, the leuco-fuchsin reagent, applied to frozen sections of skin to detect the combined aldehyde groups of plasmalogens, gave a positive reaction in Malpighian layers only.[10] In studies on the distribution of alkaline phosphatase, the Malpighian layers of the epidermis gave a slight reaction,[11–13] while those of the hair follicle gave a pronounced

---

* The authors are indebted to Dr. Phoebe Cox for suggestions and assistance in the preparation of this manuscript. The help of Miss W. F. Storey is also acknowledged.

reaction.[11, 13] The reaction was intense in those follicles where the hair was growing, but decreased, or even disappeared, where growth had ceased.[14, 15] Bulliard and Giroud,[16] using Tunnicliffe's chemical method, found 1.5 mg. of extractable glutathione per gram of fresh tissue in the Malpighian layers of the horse burr and none in the corresponding cornified layers. Thus, it is apparent from such data that many active substances are more abundant in, if not limited to, the Malpighian layers. Such findings would seem to explain the elevated metabolism of the epidermis.[17]

Chemical Similarities between Malpighian and Cornified Layers of Epidermis and Its Derivatives. Some substances may be at least partly retained in the cornified cells after completed keratinization. Glycogen has been found by Giroud, Bulliard, and Ratsimamanga in both Malpighian and keratinized layers of the horse burr (unpublished). Small amounts were indeed observed by chemical and histochemical methods in the Malpighian layers of the epidermis.[18–20] Unna[1, 21, 22] detected glycogen in the cornified layers by histochemical methods, a fact which was later confirmed by Bolliger and McDonald[23] after Sasakawa[24] had questioned Unna's claim when he, Sasakawa, could not detect glycogen in such a location. In the hair, the presence of glycogen has been reported in the outer root sheath and also in the lower part of the hair shaft near the papilla.[20–22a]

Lipids have also been found in both Malpighian and keratinized layers of "soft keratin" structures, but only in the Malpighian layer of "hard keratin" structures, FIGURE 1. Of the various chemical studies on the fat content of the epidermis,[25, 26] perhaps the most significant is that of Kooyman,[28, 27] who investigated the fat content of various types of Malpighian and cornified layers and obtained the following results:

	Phospholipids	*Cholesterol*	*Fatty acids*
Malpighian layer	2.65	1.56	3.94
Cornified layer	0.14	0.73	2.87

He concluded that the lipid and phospholipid content of the Malpighian layer, which was comparable to that of active glandular tissues, was higher than that of the cornified layer. In other words, while lipids persist through keratinization, they show some decrease, especially the phospholipids.

Histochemically, the presence of lipids may be shown with the Sudan III and IV stains in the cornified layers of the epidermis[1, 2, 22, 29] (FIGURE 1) and other soft keratin structures, such as the medulla of the porcupine quill.[30, 3] Part of these lipids must be protein-bound, since paraffin sections of the cornified layers of the epidermis, taken through the fat solvents, toluol and alcohol, can still be stained with Sudan. Rudall[30] felt that the lipids were in some way associated with the keratohyalin-eleidin system, which is involved in soft keratinization. On the other hand, in epidermal appendages consisting of hard keratin, no reaction can be obtained with Sudan stains[2, 30] (FIGURE 1).

Histochemically, certain of the amino acids making up the tissue proteins have also been investigated.[2] Thus, by means of Millon's xanthroproteic and Pauley's reactions, tyrosine has been detected in the soft keratin of the epidermis and hair medulla. Hair and other structures containing hard

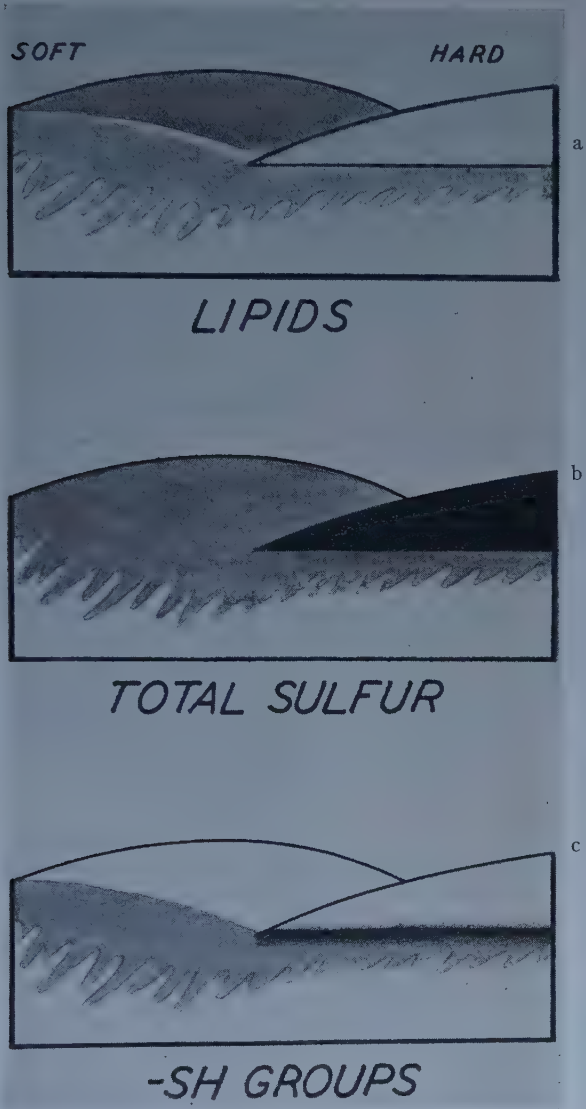


FIGURE 1. The three drawings in this figure refer to histochemical reactions on sections of the hoof of the pig's foot, the hoof itself being on the right and the sole on the left. The hoof consists of hard keratin, the sole of soft keratin. The Malpighian layers may be seen underlying both types of keratin. Diagram a is that of a section stained with Sudan III for the detection of lipids. A staining of the Malpighian layers, as well as soft keratin, is indicated. Diagram b is that of a section stained with hot sodium plumbite for the detection of total sulfur. The hard keratin stains intensely. The soft keratin and Malpighian layers stain moderately. Diagram c is that of a section stained with sodium nitroprusside for the detection of sulfydril groups. The two types of keratin remain unstained. The Malpighian layers stain moderately. At the limit between the Malpighian and cornified layers of hard keratin, there is an intensely staining band, the keratogenous zone. Presumably, the large amount of sulfur found in hard keratin comes from the oxidation of the sulfydril groups in the keratogenous zone.

keratin also react, but far more slowly, presumably due to the slower penetration of the reagents. Stoves[31] further noted that the direction of the cut of a section of hair influenced the reactivity of the cuticle and cortex. Tryptophane, as detected by the reactions of Adamkiewiez, von Furth, and Lieben and Romieu, has been found in both soft and hard keratin, but the reaction develops more slowly in hard keratin.[2] The sulfur-containing amino acids will be examined later in this paper. Histochemical results of no apparent significance in keratinization will not be mentioned in the text.*

In conclusion, it has been demonstrated that chemical similarities do exist between the Malpighian and cornified layers, in that they both contain glycogen, similar amino-acids, and lipids. Some decrease in the concentration of these substances occurs in the course of keratinization, however, especially in that of glycogen and lipids, the decrease being most pronounced with hard keratin.

Histological Similarities Between Malpighian and Cornified Layers of Epidermis and Its Derivatives: The Tonofibrils. Giroud and Champetier[42] have emphasized another similarity between Malpighian and keratinized layers: the presence of the epidermal fibrils, called "tonofibrils." These tonofibrils provide the fibrillary or structural continuity between the living Malpighian and the non-living cornified parts of the epidermal derivatives, as well as of the epidermis itself. Their presence in routinely stained slides is well known. They are easily seen by means of phase microscopy.[43] These fibrils have been shown to run from cell to cell, as well as through the cells, thus holding them firmly together.[4] In the upper Malpighian layer of the epidermis, they form a sort of network whose main direction is parallel to the surface, while at the base of an epidermal derivative, such as the hair, they tend to be definitely oriented in the line of the axis of the fully mature derivative, in this case, the hair. Weidenreich, using staining techniques, found that the cornified layers of epidermis and horny derivatives also contain tonofibrils.[44]

Panebianco[45] observed that the tonofibrils in the Malpighian layers of epidermis possess birefringent properties. Schmidt[46, 47] was able to confirm this finding in the case of hard keratin structures by demonstrating that the birefringence found in the Malpighian layer at the base of the scales of the tortoise was such as to have a doubly positive axis, and that the axial line corresponded to that of the fibrils themselves. Similarly, he showed that the birefringence in the cornified cells of the claws of the gecko was due to the tonofibrils. Mercer[48] and Schmidt,[36, 37] working with hair, found only traces of birefringence in the lower part of the hair bulb, but birefringence increased rapidly to a maximum as the "keratogenous zone" of the hair bulb was traversed and as the hair took on its own definitive characteristics. It is to be noted that, when the birefringence became

* Some histochemical investigations may be briefly mentioned:

The mineral content of the skin was studied by Dahn,[32] Farina and Sannicandro,[33] and Gans.[34] On the isolated epidermis, Suntzeff and Carruthers[35] and Cowdry, Carruthers, and Suntzeff[36] were able to estimate the mineral content, which was in mg. per cent: K, 0.322; Na, 0.122; Mg, 0.018; Ca, 0.018; and Fe, 6.4. The same authors have also demonstrated the presence of Cu and Zn in the isolated epidermis.

The melanins are recognizable histochemically by their ability to reduce ammoniacal silver nitrate[37] and the ferric-ferricyanide reagent of Chevrémont and Fréderic.[70] Generally, they are in granular form, as in deeply colored hair. But in light colored hair they are diffusely distributed. Their chemical nature is not precisely known as yet. There seems to be a chromophore component, melainine, united to amino acids, polypeptides, or proteins. The presence of iron is in doubt, except in the pigments of red hair.[38] The melanin problem is examined in a recent review.[72]

No vitamin A could be recognized in the epidermis,[39, 40] but the presence of vitamin D is indicated by an absorption maximum in ultraviolet light at 280 mμ.[41]

maximum, the tonofibrils also were well developed and parallel to one another, as well as to the longitudinal axis of the hair shaft. In conclusion, the universal presence of tonofibrils in the Malpighian and cornified layers of epidermis and its derivatives has been conclusively demonstrated by staining and birefringence methods.

By means of the electron microscope, Mercer and Rees were able to show that fibrils were present in the cortex of wool fibers (processed sheep hair) next to an amorphous matrix, composed of single aggregates of particles (and not of disorganized fibrils, as previously claimed).[49–51] By using the gold shadowing technique, it was possible to show that these tonofibrils could be further subdivided into protofibrils, the ultimate fibrous unit, which were 110 Å wide and 2000 Å long (FIGURE 2). The axes of these protofibrils appeared to be twisted, thus giving the entire tonofibril a rope-like appearance. These protofibrils, in turn, were composed of a series of particles of uniform size which had a diameter of 110 Å and which were arranged side by side along the axis.[51] It was further suggested that these particles were true keratin molecules, similar to those found in the matrix, and that the tendency they demonstrated to form fibrils may have been due to mutual attraction of the end groups of the polypeptide chains making up each molecule.[48]

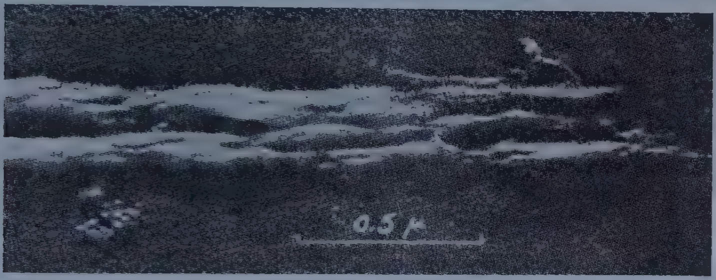


FIGURE 2. Electron micrograph of hair cortex, showing a very high magnification of two fibrils. The fibrils consist of smaller protofibrils, twisted like the fibers of a rope. The protofibrils themselves appear to be made up of granular elements. (This figure is taken from Farrant, Rees, and Mercer.[51])

In conclusion, it seems that the tonofibrils consist of twisted protofibrils, formed by addition of individual units end to end. While this finding was reported for the hair, the tonofibrils found in epidermis and other derivatives may, perhaps, consist of the same units. At any rate, the tonofibrils form the framework of all keratin structures.

X-ray Pattern Similarities between Malpighian and Cornified Layers of Epidermis and Its Derivatives. Astbury[52, 53] undertook the study of hair with the help of X-ray diffraction techniques. It is well known that a beam of monochromatic X rays directed onto a crystal will be partially diffracted by the rectilinear planes of that crystal, and that such diffracted rays can be focused and recorded on a photographic plate. Such diffracted rays produce a series of spots which can be analyzed for number, location spread, and intensity. Analysis of such data can then be used to make deductions regarding the periodicity of the structures making up the crystal.

Astbury found that human hair and sheep wool did diffract X rays as if they were crystalline, thus confirming the findings obtained by the birefringence studies. The X-ray pattern indicated that a periodicity of 5.1 Å occurred along the axis of the human hair. Since hair can be stretched 100 per cent in a warm, moist atmosphere without breaking, the X-ray pattern was examined under these conditions and a new periodicity of 3.4 Å was discovered. Later, other periodicities of higher order were also found.[54–56] Astbury suggested that the two basic periodicities were due to two forms of keratin, which he called α and β, and concluded that the α-type could be transformed into the β-type by stretching.[52, 53]

Giroud and Champetier[28] used the horse burr, a soft keratin derivative, to determine whether this material would produce the same pattern as the hard keratin of the hair. These authors separated the cornified from the Malpighian layers, stretched both slightly to line up the presumed inherent crystalline structure, and defatted and dried the material before exposing it to the incident X rays. They then found that both the cornified and Malpighian layers exhibited a periodicity of 5.1 Å, identical to the α-pattern found by Astbury in the unstretched human hair. They concluded that this α-pattern was common to hard and soft keratin, and pre-existed, as well, in the Malpighian layers, presumably as a precursor of the keratins. Derksen, Heringa, and Weidinger,[57, 58] using the snout of the cow, confirmed the presence of the α-keratin pattern in the Malpighian and soft keratinized layers, and showed that with 100 per cent stretching in warm moist conditions, a β pattern could be obtained with these structures as with the hair. Mercer[48] examined the X-ray diffraction pattern in the Malpighian layer, keratogenous zone, and cortex of the hair. The α-pattern appeared in the upper region of the Malpighian layer and persisted unchanged throughout the keratogenous zone into the hair itself (FIGURE 3). From all these results, it may be concluded that a protein with the keratin pattern pre-exists in the Malpighian zone and is actually the precursor of the true keratin formed at the limit between Malpighian and keratinized layers.

This concept was confirmed by Rudall[59] who, using a strong solution of urea, was able to isolate from the Malpighian layer of the epidermis a protein which, under proper conditions, diffracted X rays in both an α- and a β-pattern. Rudall's protein displayed long range elasticity when in fibrillary form and, in most of its properties, behaved as might be expected of a keratin deficient in cross linkages. The extractability of this protein may be noted in parallel with the fact that the tonofibrils of the Malpighian layers are easily modified by enzymes, acids, and autolytic processes, as shown by Behn,[60] Weidenreich,[44] and Patzelt.[61] In contrast, the birefringence of keratinized structures is not easily altered by these chemical treatments, indicating that tonofibrils become resistant following keratinization. Thus, Mercer showed that similar chemical treatments of longitudinal sections of hair remove the birefringence only in Malpighian layers at the level where tonofibrils begin to form (level c; FIGURE 3).[48] This work included an examination of the X-ray pattern, from which it appears that the X-ray pattern was also disorganized in the same region by the chemical treatments. Apparently, the keratinization process stabilizes both the birefringence (tonofibrils) and the molecular pattern (FIGURE 3).

These results suggest a relation between the X-ray pattern and the presence of tonofibrils, as was first indicated by Giroud and Champetier.[42] These authors also found that the α-pattern was present in the epidermis and derivatives where tonofibrils are present, but not in epithelial organs which, like the liver, do not contain tonofibrils. With these authors, it may be concluded that the tonofibrils are probably the support for the characteristic protein with an α-pattern (presumably a long-chain molecule) found in epidermis and derivatives. Further, such a protein is built up in the Malpighian layer and is presumably the keratin precursor which becomes fully keratinized in the region of passage from the Malpighian to the cornified zone, without changes in the fibrous skeleton, at least as far as the X-ray periodicity is concerned.

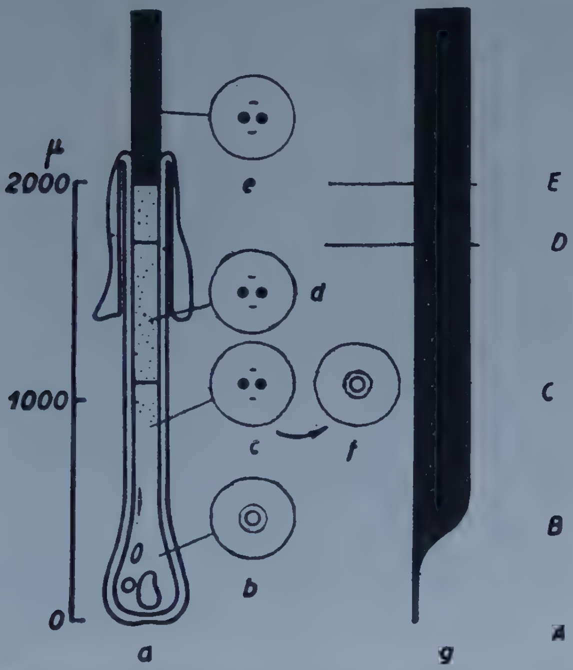


FIGURE 3. Diagram of a hair follicle on the left hand side. The circles in the center represent the X-ray diagrams at various levels of the hair follicle. The black band on the right hand side represents the intensity of the birefringence at various levels of the hair follicle. (This figure is taken from Mercer,[48] with slight modifications.) Examination of the hair follicle, starting from the Malpighian layer upwards, shows first that the deeper layers give no definite X-ray pattern and reveal only traces of birefringence (level b). Higher up, the alpha pattern of keratin appears and the Malpighian layers become birefringent (level c). At this level, however, the action of enzymes, acids, alkalis, and urea will disorganize the X-ray pattern and decrease the birefringence, unless pre-treatment has been carried out with ethylene-dibromide in the presence of sodium bicarbonate (a treatment which is known to unite reduced disulfide groups in wool). At a higher level (d), the action of enzymes, acids, alkalis, and urea does not modify the X-ray pattern and birefringence. The same is true of the hair cortex itself (level e).

Differences between Hard and Soft Keratinization: The Role of Sulfur. The differences in the lipid content of hard and soft keratin structures has been emphasized above (FIGURE 1, first diagram). Possibly, the most significant difference between the two types is found in the sulfur content and distribution. The sulfur present in epidermis and derivatives is mostly in the form of cysteine and cystine bound by peptide linkages in keratin, as well as in its precursor.

Histochemically, the presence of sulfur is easily demonstrated by using a heated sodium plumbite reagent.[2, 62] With this reagent, the "hard" keratin of horny derivatives blackens rapidly, as may be seen with horse hair, pig hoof (FIGURE 1), hedgehog quill, *etc.* Under high magnification, the reaction appears evenly distributed throughout the cells (FIGURE 4), a fact which derives added significance from the knowledge that tonofibrils are packed together throughout such cells.[4] The soft keratins, on the other hand, instead of turning black rapidly, turn brown slowly (FIGURE 1). Under high power of the microscope, the reaction was found chiefly at the periphery of the cells (FIGURE 4), which is the location, incidentally, of the tonofibrils at the time when these cells are passing through the stratum granulosum of the Malpighian layers. Little reaction was found in the stratum germinativum. The obvious conclusion is that less sulfur is present in soft than in hard keratin. In the case of hair, the medulla showed a reaction, indicating the presence of sulfur in smaller amounts than in the cortex. Bekker and King[63] concluded from their histochemical studies on wool that the medulla did not contain sulfur. However, chemical analysis of the quill of the porcupine showed a sulfur content of 1.50 per cent in the cortex, and somewhat less, 1.24 per cent, in the medulla.[2] In order to settle the question, other soft and hard keratin structures were investigated in regard to sulfur content by Giroud, Bulliard, and Giberton.[50] The sulfur

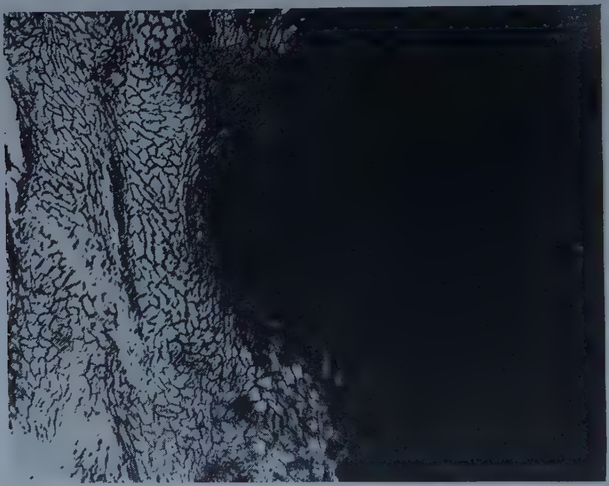


FIGURE 4. High magnification of a section of the pig's hoof stained with hot sodium plumbite for the detection of total sulfur, showing the limit between hard (right) and soft (left) keratin. The reaction of the hard keratin is an intense, solid black; that of the soft keratin is limited to the periphery of the cornified cells.

content was, in general, much higher in hard keratin (*e.g.*, 3.4 per cent of dry weight of the bull's horn) than in soft keratin (*e.g.*, 0.50 per cent of dry weight of corneum from the horse burr). The comparison of these figures with those found in Malpighian layers was also made. Thus, the sulfur made up 0.59 per cent of the dry weight of the Malpighian layer of the horse burr, in contrast to 0.50 per cent of the dry weight of the cornified layer, and, therefore, there was no increase in the sulfur content during the process of soft keratinization. In cases of hard keratinization, such as that of the bull's horn, the same authors found that the sulfur content of the Malpighian layer was 1.0 per cent of the dry weight, while that of the hard keratin was 3.4 per cent of the dry weight. Here there was a threefold increase in the sulfur content.

The differences in the sulfur content of the various types of Malpighian layers were probably not significant, since the ratio of sulfur to phosphorus (a supposedly fixed cell constituent) was similar in the Malpighian layer of horse burr (1.09) and bull's horn (1.14). Thus, the high sulfur content of hard keratins must be due to a concentration of this substance in the keratogenous zone, presumably through a loss of other material (lipids, glycogen, *etc.*) The alternative possibility of an incorporation of newly-entering sulfur compounds was, however, not eliminated.

The next step was to investigate the form in which sulfur is present in the epidermis and derivatives. The presence of sulfur as —SH or sulfydril groups (presumably in the cysteine residues of proteins) was indeed detected by the alkaline nitroprusside and the Prussian blue reagents.

By staining fresh frozen sections with the alkaline nitroprusside reagent[62, 65, 66] a technique facilitated through stabilizing the reaction by the prior addition of a zinc salt,[71] the presence of —SH groups was discovered in all Malpighian layers (FIGURE 5). The reacting material was not removed by water and hence was not the water-soluble glutathione which was also known to be present but rather an insoluble component of the tissue, presumably a protein. If, on the other hand, the fresh frozen sections were treated with salts of the heavy metals which are known to react with —SH groups, the reaction with the stabilized alkaline nitroprusside disappeared. Other possible reacting substances were eliminated by means of various controls, and, thus, this reaction could and did become specific for the cysteine residues of the tissue proteins.

The pattern of reaction found in the Malpighian layers of soft and hard keratin derivatives was found to be different. In the epidermis, the Malpighian layers reacted homogenously throughout, even in the stratum granulosum. At the outer limit of the stratum granulosum, the reaction disappeared abruptly, and, in the strata lucidum and corneum, no trace of the reaction could be found. In pathological types of keratinization, however, where the cornified layers are obviously altered, traces of this reaction could be found. In the case of hard keratin, on the other hand, an intense reaction was found in the keratogenous zone separating the Malpighian from the cornified layer (FIGURE 1, third diagram).

In hair and its epithelial sheaths, the alkaline nitroprusside reaction

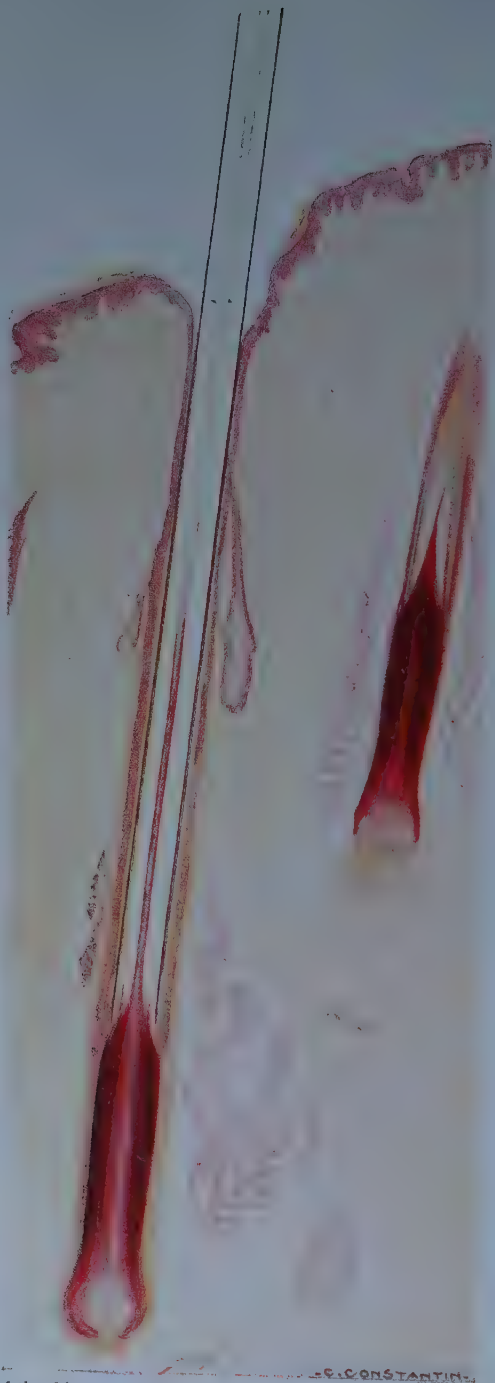


FIGURE 5. Section of the skin of a white horse, after staining with alkaline nitroprusside. A moderate reaction is apparent in the Malpighian layers of the epidermis, root sheath, and hair bulb. The soft and hard keratins do not react. In the hair being formed, a most intense reaction is visible at the keratogenous zone between Malpighian layers and hard keratin of the hair. (This figure is taken from Giroud and Bulliard.[70])

revealed a special pattern for each structure (FIGURE 5). The external root-sheath behaved exactly like the epidermis described above. The inner root-sheath, as it develops from its base toward the surface, stains slightly in its deeper portion but fails to react in its more peripheral portion. The hair bulb reacted like the Malpighian layers of the epidermis. The future medullary cells of the hair showed a similar pattern to that found in the soft keratins of the epidermis, *i.e.*, the reaction was always feeble, and faded progressively as the empty medullary cavity was approached. In contrast, in the developing future cuticle and cortex, the reaction of the Malpighian layer became progressively more intense at the point where the tonofibrils appeared, that is, somewhat deeper than the so-called "keratogenous zone." The "keratogenous zone" itself reacted intensely throughout. At its upper limit, the reaction faded out, until, in the mature cortex and cuticle, no reaction was obtained [2, 62] (FIGURE 5).

Chévremont and Fréderic[68, 69] used the ferric-ferricyanide reduction to ferro-ferricyanide, or Prussian blue reaction, to study the distribution of sulfydril groups in epidermis and its derivatives. Thin paraffin sections can be used with this reagent, which, unfortunately, is much less specific for sulfydril groups than alkaline nitro-prusside. These authors observed a reaction in Malpighian, but not in cornified layers, in confirmation of the results reported above. They claimed, however, that the intensification of the reaction found at the limit between Malpighian and cornified layers of hard keratin structures may also be observed in the comparable areas of soft keratin structures. This result may be due to the fact that the Prussian blue reaction stains other reducing substances than the sulfydril groups.

In summary, the presence of free —SH groups in the Malpighian layer and their disappearance at the points of formation of the keratinized layer seem to be well established for soft and hard keratin[2, 62, 67–69] (see also the third diagram of FIGURE 1). The —SH groups of the Malpighian layer are not extractable with water and presumably are present as cysteine residues in the protein precursor of keratin. The transformation of this precursor into keratin involves a loss of —SH groups (FIGURE 1, diagram c) but no loss or even an increase in total sulfur (FIGURE 1, diagram b). Presumably, keratinization involves an oxidation of the —SH to —S—S-groups (cysteine residues). The disulfide bonds may act as cross links between neighboring parallel polypeptide chains to form the lattice of the keratins and thus insure their strength.

From diagram b in FIGURE 1, it can also be seen that the amount of total sulfur does not change from the Malpighian to the cornified layers in the soft keratin of the pig's hoof, whereas there is a sharp increase in the hard keratin layers of the hoof proper. Presumably, hard keratin contains many more cystine radicals acting as cross links between neighboring polypeptide chains than soft keratin. This chemical distinction, as well as the abundance of lipids in the soft keratin only (FIGURE 1, diagram a) would explain the known differences in the physical and histological properties of the two types of keratin.

General Conclusions

Basic similarities have been shown to exist between structures involved in "soft" keratinization, such as the epidermis and medulla of the hair, and those involved in "hard" keratinization, such as the nails and the hair cortex and cuticle. In both types, metabolically active substances predominate in the Malpighian layers, while the cornified layers of such structures seem to be almost or completely inert.

The metabolic activity of the Malpighian layers culminates in the production of tonofibrils, morphological elements shown to be present not only in the Malpighian zone, where they are rather fragile, but also in the overlying cornified layers, where they are rather resistant.

The X-ray diffraction pattern, described by Astbury in the hair and attributed to polypeptide chains with a periodicity of 5.1 Å, can be found wherever tonofibrils are present. Furthermore, this so-called "alpha" pattern has been obtained *in vitro* with a protein extracted by Rudall from the Malpighian layers. This protein is believed to be the chief component of the tonofibrils and, as such, the keratin precursor.

The sulfur of the epidermis and its derivatives is mostly present as cysteine residues in the Malpighian layer and cystine residues in the cornified layer. At the limit between these two zones, the sulfydril group of cysteine is oxidized to the disulfide bond of cystine, a process by which the keratin precursor present in the tonofibrils of the Malpighian layer is transformed into the completed keratin present in the tonofibrils of the cornified layers. The oxidation of cysteine into cystine probably provides cross links between the individual polypeptide chains making up the tonofibrils, thus welding them into a closely knit mass. This is at least one of the mechanisms by which the strength and chemical inertness of keratin may be explained.

This process is most elaborate in the formation of "hard" keratin derivatives, such as the hair, in which there is a pronounced enrichment of sulfur at the limit between Malpighian and cornified layers. This distinction, as well as the difference in lipid content (FIGURE 1), accounts for the physical and histological peculiarities of "hard" and "soft" keratin.

Bibliography

1. Unna, P. G. 1926. Histochemie der Haut. Leipzig.
2. Giroud, A., H. Bulliard, & C. P. Leblond. 1934. Les deux types fondamentaux de kératinisation. Bull. d'histol. appliq. à la Physiol. **11:** 129.
3. Block, R. J. 1937. Chemical studies on the neuroproteins. IV. On the nature of the proteins of the ectoderm: eukeratins and pseudokeratins. J. Biol. Chem. **121:** 761.
4. Leblond, C. P. 1951. Histological structure of hair, with a brief comparison to other epidermal appendages and epidermis itself. Ann. N. Y. Acad. Sci. **53** (3): 464.
5. Stowell, R. E. & Z. K. Cooper. 1945. Relative thymonucleic acid content of human normal epidermis, hypoplastic epidermis, and epidermoid carcinomas. Cancer Research **5:** 295.
6. Felsher, Z. 1947. Studies on the adherence of the epidermis to the corium. J. Invest. Dermat. **8:** 35.
7. Biesele, J. J. 1944. Ribonucleic acid and heterochromatin in epidermal carcinogenesis. Cancer Research **4:** 737.
8. Giroud, A., C. P. Leblond, R. Ratsimamanga, & M. Rabinowicz. 1935. L'acide ascorbique ou vitamine C au niveau du tégument (derme, épiderme, cellules pigmentaires). Bull. Soc. franç. de derme, et de syph. **42:** 483.
9. Carruthers, C. & V. Suntzeff. 1942. Influence of limited application of methylcholanthrene upon epidermal iron and ascorbic acid. J. Nat. Cancer Inst. **3:** 217.

10. Voss, H. 1941. Histotopochemische Untersuchungen über des Verhalten der menschlichen Haut zur Plasmalreaktion. Deutsches med. Wchnschr. **67:** 127.
11. Fisher, I. & D. Glick. 1947. Histochemistry; localization of alkaline phosphatase in normal and pathological human skin. Proc. Soc. Exper. Biol. & Med. **66:** 14.
12. Bourne, G. 1943. The distribution of alkaline phosphatase in various tissues. Quart. J. Exper. Physiol. **32:** 1.
13. Fell, H. B. & J. Danielli. 1943. The enzymes of healing wounds. I. The distribution of alkaline phosphomonoesterase in experimental wounds and burns in the rat. Brit. J. Exper. Path. **24:** 196.
14. Johnson, P. L., E. O. Butcher, & G. Bevelander. 1945. The distribution of alkaline phosphatase in the cyclic growth of the rat hair. Anat. Rec. **93:** 355.
15. Bevelander, G. & P. L. Johnson. 1947. Phosphotase glycogen relationships in integumentary derivatives. Anat. Rec. **97** (Suppl.): 322.
16. Bulliard, H. &. A. Giroud. 1929. Peau et glutathion. Ann. de dermat. et syph. **10** (Ser. 6): 73.
17. Wohlgemuth, J. & E. Klopstock. 1926. Die Fermente der Haut. Atmung und Glykolyse der Haut und ihre Beeinflussung durch Hormone. Biochem. Ztschr. **175:** 202.
18. Lubarsch, O. 1924. Handbuch der Speziellen pathologischen Anatomie und Histologie. Springer. Berlin.
19. Mancini, R. E. 1946. Histochemical study of glycogen in tissues. Anat. Rec. **101:** 149.
20. Johnson, P. L. & G. Bevelander. 1946. Glycogen and phosphatase in the developing hair. Anat. Rec. **95:** 193.
21. Unna, P. G. 1913. Biochemie der Haut. G. Fischer. Jena.
22. Unna, P. G. & L. Golodetz. 1909. Die Hautfette. Biochem. Ztschr. **20:** 469.
22a. Leblond, C. P. 1950. Distribution of periodic acid-reactive carbohydrates in the adult rat. Am. J. Anat. **86:** 1.
23. Bolliger, A. & N. D. McDonald. 1949. Histological investigation of glycogen in skin and hair. Austral. J. Exper. Biol. **27** (2): 223.
24. Sasakawa, M. 1921. Beiträge zur Glykogenverteilung in der Haut unter normalen und pathologischen Zuständen. Arch. f. Dermat. u. Syph. **134:** 418.
25. Eckstein, H. C. & U. J. Wile. 1926. The cholesterol and phospholipid content of the cutaneous epithelium of man. J. Biol. Chem. **69:** 181.
26. Engman, M. F. & D. J. Kooyman. 1934. Lipids of the skin surface. Arch. Dermat. & Syph. **29:** 12.
27. Kooyman, D. J. 1931–32. Relation of the lipids to physiological activity changes in lipids of the epidermis during keratinization. Proc. Soc. Exper. Biol. & Med. **29:** 485.
28. Kooyman, D. J. 1932. Lipids of the skin. Some changes in the lipids of the epidermis during the process of keratinization. Arch. Dermat. & Syph. **25:** 444.
29. Nicolau, S. 1911. Recherches histologiques sur la graisse cutanée. Ann. dermat. et syph. **2** (Ser. 5): 641.
30. Rudall, K. M. 1947. X-ray studies of the distribution of protein chain types in the vertebrate epidermis. Biochem. et Biophys. Acta **1:** 549.
31. Stoves, J. L. 1945. Histochemical studies of keratin fibres. Proc. Roy. Soc. Edinburgh **62** (Ser. B): 132.
32. Dähn, W. 1926. Ueber die Kalzium- und Kaliumvirtilung in der normalen Haut. Dermat. Wchnschr. **82:** 425.
33. Farina, S. & U. Sannicandro. 1930. Calcio e potassio della cute. Gior. ital. di dermat. e sifil. **71:** 1360.
34. Gans, O. 1930. Zur Histo-Topochemie der gesunden und Kranken Haut. Untersuchung des anorganischen Aufbaues mittels der Schnittveraschung. Arch. f. Dermat. u. Syph. **161:** 607.
35. Suntzeff, V. & C. Carruthers. 1945. The mineral composition of human epidermis. J. Biol. Chem. **160:** 567.
36. Cowdry, E. V., C. Carruthers, & V. Suntzeff. 1948. Influence of age on the copper and zinc content in the epidermis of mice undergoing carcinogenesis with methylcholanthrene, and a note on the role of calcium. J. Nat. Cancer Inst. **8:** 209.
37. Lison, L. 1936. Traité d'histochimie animale; méthodes et problèmes. Gauthier-Villars. Paris.
38. Flesh, P. & S. Rothman. 1945. Isolation of an iron pigment from human red hair. J. Invest. Dermat. **6:** 257.
39. Cornbleet, T. & H. Popper. 1942. Properties of human skin revealed by fluorescence microscopy. The normal skin; the vitamin A content of the skin Arch. Dermat. & Syph. **46:** 59.

40. Popper, H. 1941. Histologic distribution of vitamin A in human organs under normal and under pathologic conditions. Arch. Path. **31:** 766.
41. Bachem, A. 1929. Ultraviolet transparency of the various layers of human skin. Proc. Soc. Exper. Biol. & Med. **26:** 778.
42. Giroud, A. & C. Champetier. 1936. Recherches sur les roentgénogrammes des kératines. Bull. Soc. Chim. de biol. **18:** 656.
43. Albertini, A. 1945. Pflastorepithelizellen im Phasenkontrastbkld. Acta anat. **1:** 463.
44. Weidenreich, F. 1900. Ueber Bau und Verhornung der menschlichen Oberhaut. Arch. f. mikr. Anat. **56:** 169.
45. Panebianco, E. 1897. Studio ottico cristallografico della cheratino. Riv. di Mineral. cristalogr. ital. **17:** 1.
46. Schmidt, W. J. 1924. Die Bausteine des Tierkorpers im polarisiertem Lichte. Bonn.
47. Schmidt, W. J. 1934. Handb. d. biol. Arbeits Method. **10** (Abt. 5): 435.
48. Mercer, E. H. 1949. Some experiments on the orientation and hardening of keratin in the hair follicle. Biochem. & Biophys. Acta **3:** 161.
49. Mercer, E. H. & A. L. G. Rees. 1946. An electron microscope investigation of the cuticle of wool. Austral. J. Exper. Biol. **24:** 147.
50. Corter, C. J. & A. L. Houwink. 1948. On electron microscope study of the structure of the woolhair. Proc. Amsterdam **51:** 262.
51. Farrant, J., A. L. G. Rees, & E. H. Mercer. 1947. Structure of fibrous keratin. Nature **159:** 535.
52. Astbury, W. T. 1933. Tr. Faraday Soc. **29:** 193.
53. Astbury, W. T. & H. J. Woods. 1934. X-ray studies of the structure of hair, wool, and related fibres. II. The molecular structure and elastic properties of hair keratin. Philosoph. Tr. Roy. Soc. London **232** (Ser. A): 333.
54. MacArthur, I. 1943. Structure of α-keratin. Nature **152:** 38.
55. Rudall, K. M. 1947. X-ray studies of the distribution of protein chain types in the vertebrate epidermis. Biochim. & Biophys. Acta **1:** 549.
56. Bear, R. S. & H. J. Rugo. 1951. The results of X-ray diffraction studies on keratin fibers. Ann. N. Y. Acad. Sci. **53** (3): 627.
57. Derksen, J. C. & G. C. Heringa. 1936. Polska gaz. Lekarska **15:** 532.
58. Derksen, J. C., G. C. Heringa, & A. Weidinger. 1937. On keratin and cornification. Acta neerland. Morph. **1:** 31.
59. Rudall, H. R. 1946. Structure of epidermal protein. Symposium on fibrous protein. J. Soc. Dyers & Colourists : 15.
60. Behn, D. 1892. Verhornung der menschichen Oberhaut. Arch f. mikr. Anat. **39:** 581.
61. Patzelt, V. 1926. Zum Bau der menschen Epidermis. Ztschr. f. mikr. Anat. Forschung. **5:** 371.
62. Giroud, A. & H. Bulliard. 1930. La kératinisation de l'épiderme et des phanères. Genèse des substances soufrées de la kératins. Arch. morph. gén. et expér. (fasc. 29) : 1.
63. Bekker, J. G. & A. T. King. 1931. Sulphur distribution in the component structures of wool and porcupine quills. Biochem. J. **25:** 1077.
64. Giroud, A., H. Bulliard, & A. Giberton. 1929. Y-a-t-il une accumulation élective de soufre précédant la kératinisation? Compt. rend. Soc. de biol. **101:** 1024.
65. Kaye, M. 1924. Observations on the behaviour of a substance giving the nitroprusside reaction in skin and in hair. Biochem. J. **18:** 1289.
66. Walker, E. 1925. The sulphydryl reaction of skin. Biochem. J. **19:** 1085.
67. Percival, G. H. & C. P. Stewart. 1930. On the sulphydryl-containing constituent of the epidermis and its relationship to melanogenesis and keratinization. Brit. J. Dermat. **42:** 215.
68. Chevrémont, M. & J. Fréderic. 1943. Une nouvelle méthode histochimique de mise en évidence des substances à fonction sulfhydrile. Application à l'épiderme, au poil et à la levure. Arch. de biol. **54:** 589.
69. Fréderic, J. 1949. Etude histologique et histochimique de la peau de cobaye traitée par les rayons X. Arch. de biol. **60:** 79.
70. Giroud, A. & H. Bulliard. 1935. Les substances à fonction sulfhydryle dans l'épiderme. Arch. d'anat. micr. **31:** 271.
71. Giroud, A. & H. Bulliard. 1932. Sur une réaction des substances à fonction sulfhydryle. Bull. Soc. de Chim. biol. **14:** 278.
72. Lerner, A. B. & T. B. Fitzgerald. 1950. Biochemistry of melanin formation. Physiol. Rev. **30:** 91.

THE RESULTS OF X-RAY DIFFRACTION STUDIES ON KERATIN FIBERS

By Richard S. Bear and Henry J. Rugo*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Polypeptide Chain Configurations in Keratin Fibers. Animal hairs and other keratinous tissues have occupied a central place in the study of the fine structure of protein fibers. Much of this development is due to British investigators, who have introduced concepts and terminology widely encountered in the literature. The roles of side chains and backbones in the folding and unfolding of polypeptide chains are now almost classical parts of the thinking regarding these systems.

According to the views of the British school, the polypeptide molecular chains of the better arranged (crystalline or fibrillar) portions of many fibers may exist in three principal stages of contraction or extension, each characterized by a distinctive X-ray diffraction diagram (Astbury and Bell, 1939). These diagrams have been interpreted, in relation to other physical and chemical observations, as indicating that the following are the three principal structural configurations available to the polypeptide chains (FIGURE 1). Normally, in such structures as mammalian hairs, the chains are in the somewhat contracted or α form. Under certain conditions, notably at elevated temperatures in aqueous environments, the application of longitudinal tension results in an elongation (70 to 100 per cent), with transformation to an extended or β state. In similar environments (with possibly the help of reducing and/or hydrogen-bond relaxing agents), complete release of tension will eventually result in a third, or supercontracted, state, in which the fiber has contracted to considerably less than the original length (*ca.* 30 per cent).

Early studies showed that supercontracted fibers may be in a condition exhibiting disoriented, β-type diffractions. Subsequently, Rudall (1946), working largely with epidermal material, concluded that the chains are often thrown into long folds of specific type, the loops of which extend normal to the fiber axis (an exaggeration of the folds of α type shown in FIGURE 1a). The arms of the folds develop a structure much like the extended (parallel-β) form, except for predominant orientation of the main-chain segments normal to the fiber axis (the cross-β configuration).

Rudall found that saturated urea solution at room temperature is a particularly useful reagent for securing, under appropriate conditions of tension, reversal of the cross- and parallel-β forms back to the original α condition. Transformations of this sort, however, become more difficult if the fiber has previously been held in steam at a given length, allowed to relax its tension spontaneously, and then permitted to become set in the new configuration.

Speakman (1947), in particular, has defended the view that the processes of relaxation and set involve chiefly the interaction of side chains. During

---

* National Cancer Institute Research Fellow in the Department of Biology, 1947–1948, the period of the studies on feather keratin herein reported.

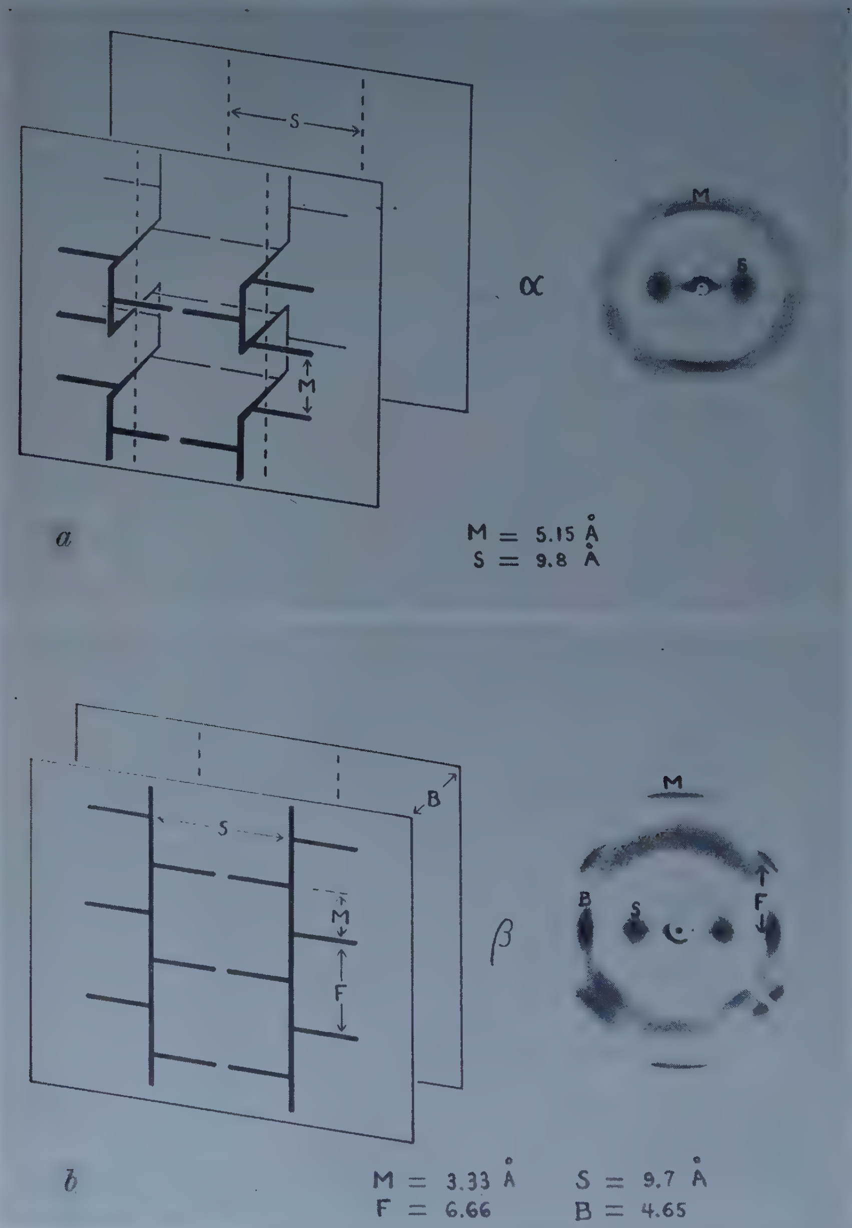


FIGURE 1. Comparison of the Astbury models for α and β structures of the fibrous proteins with the corresponding wide-angle diffraction diagrams. The models are diagrammatic, with the solid lines, which run in a general vertical direction, representing the main polypeptide chains, —(—CHR -CO -NH—)$_n$—; the horizontal lines depict the extended side-chain (R) groups characterizing the different amino-acid residues. Planes of the models run through the centers of gravity of grids which parallel the general main-chain and side-chain directions. Capital letters show the relation between model spacings and the corresponding diffractions as follows: S, side-chain spacings; B, backbone spacings; M, prominent, vertically oriented, interside-chain spacings; and F, the main-chain fiber period of the β structure.

relaxation, the stabilizing unions between the side chains, especially the disulfide links, which help to preserve a pre-existing main-chain fold, become hydrolytically or otherwise cleaved to relieve stresses, while their subsequent reunion in new ways establishes or sets the new condition. Elöd and Zahn (1949a) and Alexander (1949) have defended the significance of hydrogen bridges, between C=O and N—H groups of adjacent backbone segments, in these phenomena. The latter finds solutions of LiBr particularly useful in investigating the importance of these bridges (see the article of Alexander elsewhere in this monograph).*

Whewell and Woods (1946) have described a totally different temporary type of supercontraction, brought about by immersing wool fibers in cupra-ammonium hydroxide at room temperature. In this case, addition of mineral acid spontaneously reverses the contraction to accomplish regain in length and return of the α diagram. The copper is believed to complex with somewhat separated groups along the polypeptide, bringing them closer during the contraction without permanently disturbing the inherent α fold. The normal α diagram disappears during the contraction without being replaced by a new set of distinct diffractions.

The most recent models proposed by Astbury (1941) have been designed to satisfy the more obvious criteria regarding the magnitudes and relative orientations of the X-ray spacings (FIGURE 1), the requirements of space-filling (density, adequate room for side chains, *etc.*), and the phenomena of extensibility described above. Undoubtedly, the proposed β model is most satisfactory in these respects, since more or less direct evidence has been secured in a number of ways.

The close relation of the β model to the structure of silk fibroin helps to establish the significance of the fiber period. The β-type protein fibers normally yield fiber periods (6.7 Å. in stretched hair; 6.2 Å. in feather keratin) which are somewhat shorter than the 7.0 Å. observed with silk. Half of the silk period (3.5 Å.) has been considered the maximum extension permitted for an amino-acid residue along the polypeptide chain (Meyer and Mark, 1928). Corey (1940) estimated, however, that the effective length per residue in a completely extended polypeptide chain could be 3.67 Å., basing this figure on data obtained from structure determinations for glycine, alanine, and diketopiperazine. Astbury, Dalgliesh, Darmon, and Sutherland (1948) have reported indications that the effective residue length may be somewhat greater than the 3.5 Å. of silk in the synthetic polypeptides, polyglycine, and poly-DL-alanine. In general, it would appear that the larger residue extensions are obtained when side chains are relatively small, and hence provide less resistance to full main-chain extension.

Comparisons of the structures of β keratin, silk, and certain polyamides (Brill, 1943; Bunn and Garner, 1947) establish fairly well the general interpretation of the backbone direction. These studies, in conjunction with production of β-keratin specimens with higher orientation, induced by compression transverse to the fiber axis (Astbury and Sisson, 1935), show that, in actual fact, the side-chain and backbone spacings are mutually

* P. 653.

orthogonal, or nearly so. In such instances, as was also found from direct measurement of built-up films formed from egg albumin monolayers (Astbury, Bell, Gorter, and Van Ormondt, 1938), the side chains extend normal to the film or compressed sheet surface. Results of this type have indicated the stability of planes of polypeptide chains held together by hydrogen bridges in the backbone direction (Elöd, 1941), in contrast to earlier models which (as in FIGURE 1) tended to emphasize laminar grids established by side-chain interactions (*cf.*, however, Astbury, 1940).

The α model may be subject to further revision (Taylor, 1941; Huggins, 1942, 1943; Bull and Guttman, 1944; Zahn, 1947; Ambrose and Hanby, 1949), but it seems difficult to be more detailed until the data, notably those supplied by diffraction study, permit improvement. In particular, the suggestions of Lotmar and Picken (1942) can not become convincing until their results are made reproducible and are proved related to a protein component (of muscle, in their instance).

One of the directions from which new information may be expected is furnished by the recently available synthetic polypeptides. These have already aroused the interest of a number of investigators (Astbury, Dalgliesh, Darmon, and Sutherland, 1948; Ambrose and Hanby, 1949; Brown, Coleman, and Farthing, 1949; Bamford, Hanby, and Happey, 1949). While no conclusive results have yet been obtained, it is already clear that these substances, whose population of side chains is controllable and may be kept quite simple as compared to natural proteins, will be capable of providing more definitive diffraction evidence than has been available regarding the configurations open to polypeptide molecular chains. Some synthetic materials possess structures resembling the α or β type of configuration, but conversion from one type to the other has not been accomplished with any single preparation.

An outstanding difference between the keratinous fibers and the highly elastic polymers of rubber-like character lies in the manner of maintenance of tension. Bull (1945), Woods (1946), and Elöd and Zahn (1949b) have shown that, in hair and wool fibers, the tension developed from a tendency of chain segments to gain entropy by assuming random orientation under thermal agitation (as is the case with rubber) is often small in comparison to the larger contribution resulting from an inclination to adopt specific contracted configurations. This is in line with the general picture for the protein fibers as outlined above. These views have been challenged recently by Meyer and Haselbach (1949), though without conspicuous success (Woods, 1949).

Whatever the eventual fate of the specific α and β models defended by Astbury and his colleagues, the experimental background they have developed furnishes a central body of information of lasting significance for the understanding of the structure of protein fibers. This is particularly apparent in the demonstrations that the diffraction patterns of hair and wool fibers, and their transformations, are exhibited, with minor differences, by many other proteins.

Astbury has classified the native fibrous proteins, on the basis of their diffraction effects, into two great categories: the collagen class (*cf.* Marks, Bear, and Blake, 1949) and the keratin-myosin-epidermis-fibrinogen or, more briefly, the keratin-myosin class (Astbury, 1947). The latter group is of primary concern to the present discussion, and, as the name implies, it comprises the hard and soft keratinous products of mammals, amphibians, fishes, birds, and reptiles; the muscle fibers of all forms; and blood fibrinogen, as well as the fibrin derived therefrom. The soft epidermal layers, of all of the animal classes mentioned, normally exhibit α diffraction diagrams, also exhibited by the hard keratins (hairs, quills, nails, claws, horns, whalebone, *etc.*) of mammals. The hard keratins (feathers, scales, scutes, beaks, and claws) of birds and reptiles, however, occur naturally, with a structure resembling that of the β configuration (see references below to feather keratin). The details concerning the distribution of the various keratin types among vertebrate animal tissues have been studied by Giroud and Bulliard (1930; see the paper of Giroud and Leblond elsewhere in this monograph*) and by Rudall (1947).

The validity of the diffraction classification of protein fibers would seem to be independent of any particular models adopted for the corresponding polypeptide chain configurations, since diffraction patterns may be regarded as furnishing empirical criteria for structural differences and similarities of the protein fibers. This seems to have been misunderstood in the recent criticism by Meyer (1949), who has been answered by Astbury (1949). Further considerations regarding relationships within the keratin-myosin class are brought out below.

The Astbury models are also of significance for the large body of corpuscular proteins which, because of ready dispersion into globular molecular particles, are normally considered non-fibrous, though crystallizable. These are transformed into a parallel, cross, or disoriented β condition upon denaturation, depending on whether sufficient tension is introduced to accomplish full, partial, or no orientation or extension of the polypeptide chains (Astbury and Lomax, 1935; Astbury, Dickinson, and Bailey, 1935; Palmer and Galvin, 1943; Senti, Eddy, and Nutting, 1943; Senti, Copley, and Nutting, 1945; Nutting, Halwer, Copley, and Senti, 1946; Rudall, 1946). Because of the relation of the β diagram to models containing parallel straight segments of polypeptide chains, it has often been presumed that the above facts are evidence that denaturation of the corpuscular proteins is attended by an unrolling process which alters the molecules into long chain-like threads. These, by lateral aggregation, take on a fibrous form with β-type structure.

While this could often be the case, instances are known in which corpuscular molecules may aggregate directly into fibrous form without the unrolling process. Waugh (1948) has been able, for example, to transform corpuscular insulin into fibrous form without significant alteration of the molecules, as is shown by the ease with which the reverse transformation is accomplished to yield biologically active, corpuscular material once again.

Of direct bearing on the keratin-myosin class of fibrous proteins are the results with the actin of muscles, which readily undergoes a globular-fibrous transformation (Straub, 1943) that can be followed with the electron microscope (Jakus and Hall, 1947; Astbury, Perry, Reed, and Spark, 1947; Rozsa, Szent-Gyorgyi, and Wyckoff, 1949). Bailey (1949) has described tropomyosin, which he regards as a large globular monomer of myosin, suggesting also that similar particulate units are present in the keratins as well (see also Astbury, Reed and Spark, 1948). Indeed, Farrant, Rees, and Mercer (1947) believed that, with the electron microscope, they could detect such particles, of the average diameter of 110 Å., in the ultramicroscopic fibrils present in hair cortical cells.

---

* P. 613.

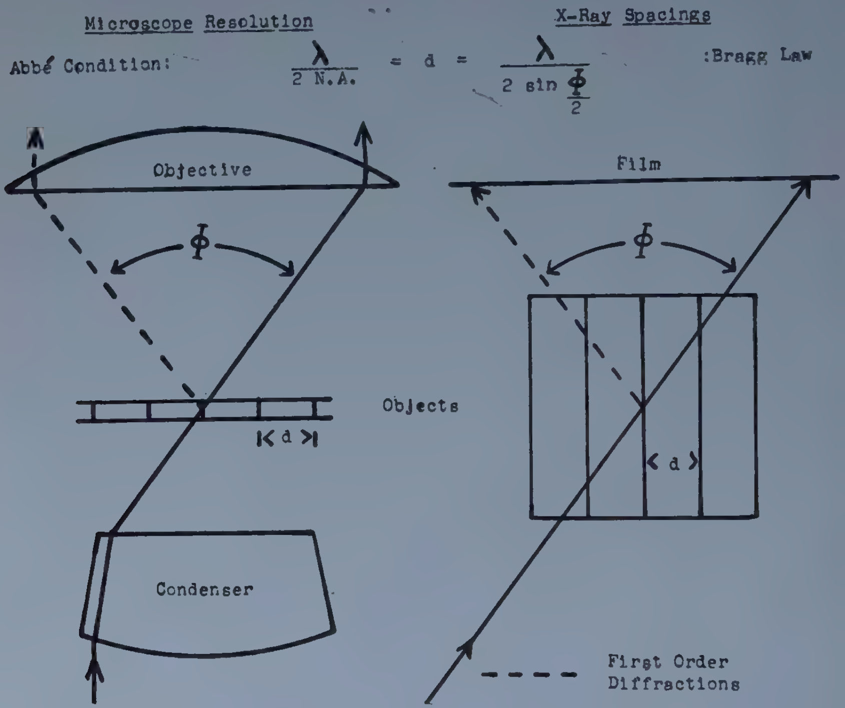


FIGURE 2. A comparison of the microscopic (left) and X-ray diffraction (right) methods for the resolution of the structural spacings, *d*, of materials. The solid line in each case represents the undiffracted or dioptric beam, and the dotted line is the first-order diffraction. The Abbé condition for resolution of *d* with the microscope is that the optical system must possess sufficient numerical aperture [N.A. = μ sin ($\Phi/2$), where μ is the refractive index of the immersion medium] to include at least the first-order diffraction. In the X-ray case, the separation *d* is that between like planes of atoms in a crystal, but, since lenses are not available, the diffractions, rather than a reconstituted image, are directly registered. Note that in both cases *d* is reciprocal to sin $\Phi/2$. In actual diffraction work, the registering film is normal to the undiffracted beam.

It is in connection with phenomena of the sort just described that prevalent models of protein fiber structure are most inadequate at the present time. This paper attempts to show in a preliminary way how X-ray diffraction studies may contribute to the solution of the difficulty, making use, chiefly, of information regarding the keratinous fibers.

Small-Angle Diffraction by Keratin Fibers. The diffraction method is, in effect, an extension of microscopy to wave-lengths of radiation which are too small to be used readily for direct image formation. FIGURE 2 shows, for example, a comparison between the well-known Abbé condition for microscope resolution and the Bragg law, which is the basis for the measurement with X-rays of the spacings between similar planes of ordered (crystalline) substances. It will be seen that the numerical aperture of the microscopist is the direct analogue of the diffraction angle of the X-ray crystallographer.

There is a curious difference, however, between the photomicrograph and the X-ray diffraction pattern, outside of the fact that only in the former is the enlarged image of the object directly recorded. It is relatively easy in microscopy to make use of the small-aperture diffractions (with low-power lenses) to obtain images of the large structural details. On the other hand, because the diffraction pattern is, in a sense, a reciprocal image, the wide-angle diffractions are most easily separated from the undiffracted or dioptric beam, so that small structure spacings are most readily investigated by means of X-rays.

This inversion of structure by the diffraction pattern has caused, in protein fiber work, a corresponding reversal in the normal time sequence with which the various levels of structure size have been studied. The smallest details have been, instead of the most difficult and last, the easiest and first to be investigated. Indeed, the general concepts of protein fiber structure currently in use, as outlined above, are largely based on the early results at wide diffraction angles; the more recent findings at small diffraction angles have had little influence as yet.

If the protein fibrils are indeed compounded of large globular monomers or molecular particles, evidence of this fact should appear at small diffraction angles. That sharp diffraction effects of this type are exhibited by keratin fibers has been known for some time, ever since Marwick (1931; see also Astbury and Marwick, 1932; Astbury, 1947) first described the well-developed pattern yielded by feathers. Later, Corey and Wyckoff (1936) obtained information for both feather and porcupine quill. That animal hairs also are capable of small-angle diffraction was noted by MacArthur (1943). He found, however, that much the best diagrams could be obtained from the tips of porcupine quills, which may be regarded as specialized mammalian hairs and more suitable for diffraction studies than are other α-keratin structures. FIGURE 3 compares porcupine quill and human hair by means of patterns secured by the present authors. The differences disclosed in TABLE 1 are no greater than might result from slight variations in extension.

The small-angle data which may eventually be accumulated for hair and porcupine quill give promise of being great, but up to this moment, this has been disappointing. It has been fairly conclusively demonstrated, both by MacArthur and by Bear (1943), that the smallest necessary fiber period of the quill is normally 198 Å., though the former at one time wished, for reasons now outmoded, to favor a considerably higher value, 658 Å. There also appear on the equator of the pattern (horizontal midlines in the reproductions of FIGURE 3) several diffractions which are at least approximately accounted for as the first three diffraction orders of an 83 Å. spacing transverse to the fiber axis, though whether they actually may be so interpreted remains an uncertainty.

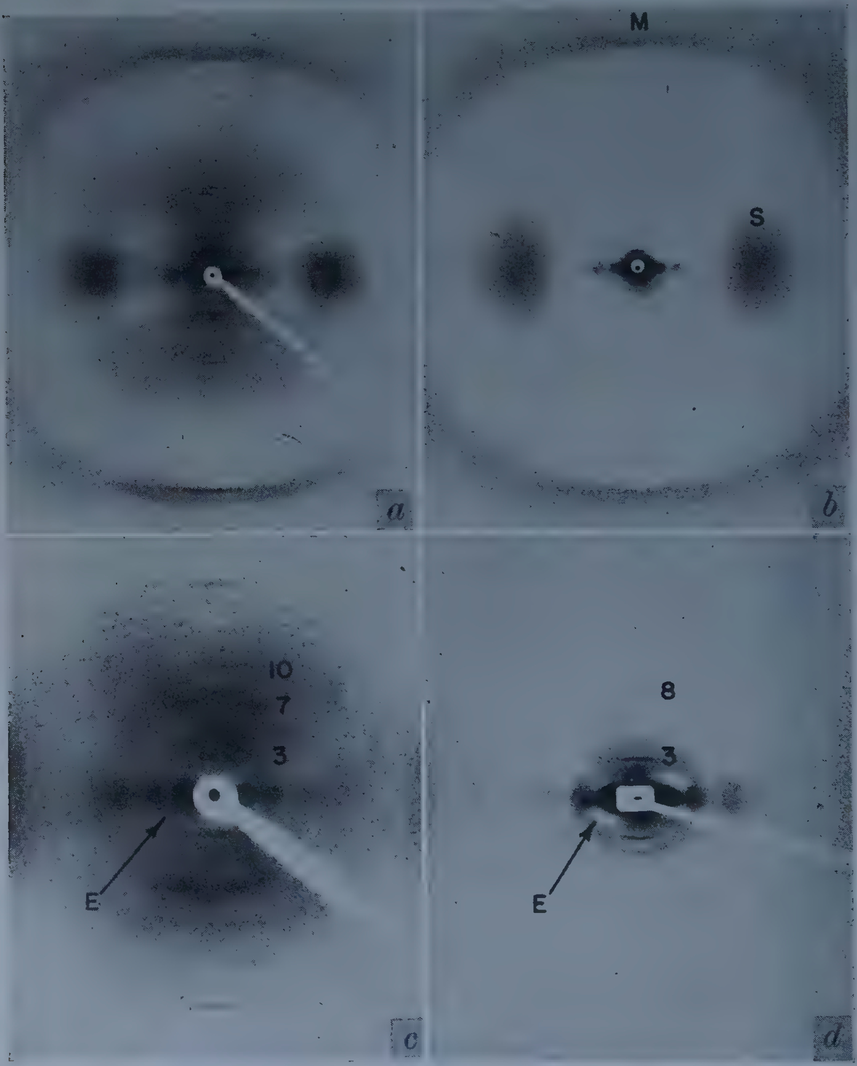


Figure 3. Diffraction diagrams of African porcupine quill and of human hair: a and b are the respective wide-angle diagrams, and c and d the small-angle patterns in the same order. The fiber axis is vertical in all cases. The wide-angle diffractions are labelled like the α diagram of figure 1, while in the small-angle patterns E indicates the strong equatorial spot (80–90 Å.) and the numbers indicate the outstanding meridional diffractions in terms of their k indices (*cf.* table 1).

Among the keratin fibers, feather specimens offer the most hope for successful study and, indeed, this material seems strategic for a number of reasons: (1) the wide-angle aspect of the X-ray diagram is of β type, which, as mentioned above, has been interpreted upon a firmer basis than the α diagram of mammalian fibers; (2) the fiber period (95 Å.) and a prominent transverse spacing (34 Å.) are about as small as can be observed for protein fibers (Bear, 1944), giving promise of relatively small unit cells; and (3), perhaps because of the small cell size, the small-angle and wide-angle diffractions merge one into the other, suggesting that with feather it may be possible to extend understanding of the relation between the two size ranges. In other protein fibers, there is a considerable hiatus between the colloidal and the finer details. FIGURE 4a shows the entire wide-angle and small-angle diffraction pattern of feather, from which it is clear that the diffraction field is quite rich and can be studied with fair accuracy.

TABLE 1

SMALL-ANGLE DIFFRACTIONS OF α-KERATIN FIBERS

Meridional and near-meridional reflections

k	*African porcupine quill*			*Human hair*		
	d(Å)	b_o(Å)	I	d(Å)	b_o(Å)	I
3	66	198	6	65	195	6
4	49*	196	1	49*	196	1
5	39	195	2			
7	27.4	192	4	27.6	193	1
8	24.5	196	2	24.3	194	4
9	22.0	198	2			
10	19.8	198	4	19.4	194	2
11	18.1	199	3			
13	15.2	198	1	15.0	195	2
15	13.2	197	1			
16	12.4	198	4	12.1	194	2
19	10.4	198	3			
Equatorial reflections						
h(?)		a_o(?)			a_o(?)	
1	83	83	10	90	90	10
2	45*	90	6	47*	94	6
3	28	84	4	29	87	4

The d columns contain the measured spacings which, when multiplied by the assigned k or h indices, yield the large fibril period, b_o, or the large transverse fundamental spacing, a_o, of 80–90 Å. The I columns indicate in rough fashion the relative intensities of the diffractions.

* Overlaid with faint, poorly oriental rings, probably due to lipid.

Bear (1944, 1945) compared the small-angle diagrams of several members of the keratin-myosin group: feather, porcupine quill, and two types of muscle fibril (later designated as paramyosin and myosin by Schmitt, Bear, Hall, and Jakus, 1947). By this comparison it was disclosed that members of this group (porcupine quill and the muscle fibrils), whose patterns are quite similar at wide angles (α-type), by no means approach identity of diffraction at small angles.

It was not concluded, however, that the small-angle results destroy the value of the Astbury classification. Rather, they tend to illuminate its significance, as may be seen by comparing the keratin-myosin fibers with

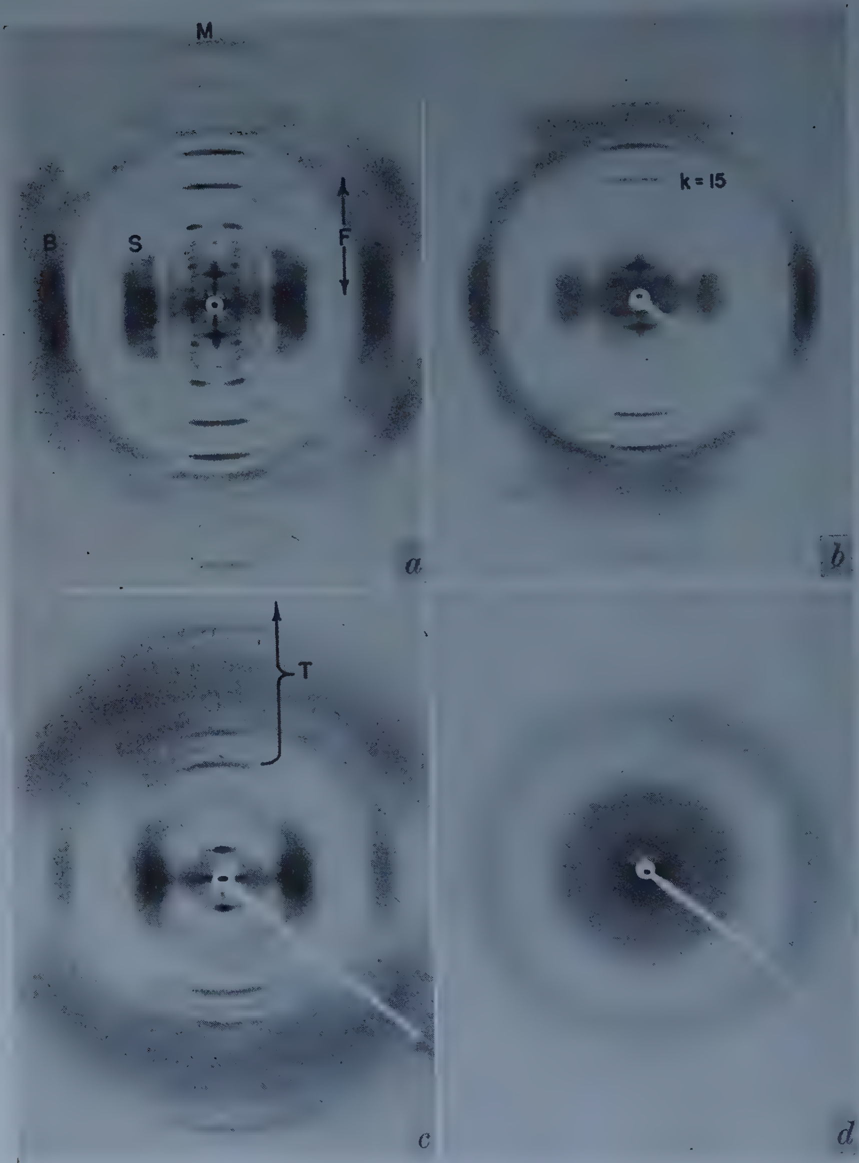


Figure 4. Various complete diagrams for sea gull feather specimens. That normally obtained from air-dried, untreated specimens is shown in a. The patterns b, c, and d represent various stages in the degradation of structure produced by "heat-moisture" treatment, these examples having been derived in the following ways: b, heating at 120° C. for 15 min. in aqueous butanol (saturated at room temperature); c, heating in water at 100° C. for 4 hours; and d, heating in distilled water for 35 min. at 120° C. All patterns are at the same magnification, with fiber axes vertical. Wide-angle identification of diffraction is facilitated in pattern a by use of the symbols of figure 1. The transitional lines on the meridian are indicated by T on pattern c, while on pattern b is shown the position $k = 15$, referred to in the text.

Astbury's other great class, the collagen fibers. Members of the former group possess large fibril periods of greatly differing magnitudes and also characteristic large spacings transverse to the fibril axis. In contrast, the collagens exhibit only a large fibril period, variations in the size of which are insignificant across wide phylogenetic excursions (Mark, Bear, and Blake, 1949).

While porcupine quill and feather represent, respectively, the α and β subgroups of the keratin-myosin family, it does not follow that their small-angle diffractions should be transformable one into the other by the manipulations which convert α to β and *vice versa* at wide angles. It is characteristic of members of this group that their small-angle diffractions vary with the source; indeed, the large structures are quite sensitive to manipulations which may destroy or profoundly disturb them. It is rather to be expected that information gained about one member will throw a general, but not detailed, light on the structure of the others.

Effects of Heat, Moisture and Alcohol Sorption on Feather Diffraction. Methods of studying protein fibers at small diffraction angles have recently been summarized (Bolduan & Bear, 1949) and need not be repeated here. Results of a previous study of strips of feather rachis (sea gull) have been described (Bear, 1944) for material equilibrated with an atmospheric environment at room temperature. Additional information, accumulated when feather specimens of the same type were subjected to certain variations in their physical and chemical condition or environment (Rugo, 1949), is presented on this occasion, whereupon alterations in the observed diffraction patterns were obtained which are significant for the present discussion.

The feather diagram is very complex, which is desirable from the standpoint that it presents a wealth of diffraction data. Unfortunately, however, all of the diffractions can not be measured with the accuracy that may be desired, for many purposes, because of spot diffuseness which increases as one departs from the pattern center, particularly away from the meridian. During manipulative experiments, the large fiber period of about 95 Å. remains relatively constant, but the row lines show displacements transverse to the meridian and/or disintegration of sharpness, in characteristic ways. If a specimen has not been altered by treatment in the same way throughout its volume, the result may be a complicated superposition of details, characteristic of different portions of the specimen. For these reasons, it is often difficult to present a complete description of a given diffraction situation or to be sure that all details of a diagram are significant for a single normal or altered structure. Consequently, in this initial presentation of the results of a variety of observations, only broad description will be made by means of statements which seem fairly definitely possible at the moment.

The two general observations which are of interest to the present discussion are derived from: (1) the production of what will be termed a net diagram by a number of means, and (2) the general effects of sorption by the feather specimens of small molecules, such as water and several alcohols.

The result of one of the better means of producing the net diagram is reproduced in some of the diagrams of FIGURES 4 and 5. A series of degradations of the feather structure was accomplished by application of heat in the presence of a saturated aqueous butanol solution. After varying lengths of exposure, the specimens were air dried and photographed at room temperature. Starting with 4a or 5b, the diagram characteristic of untreated air-dried feather, a number of the diffractions fade and others increase in intensity until the net diagram, 4b or 5d, is obtained. This, too, is eventually simplified until patterns like 4c or 5f are obtained, in which are left, near the diagram center, the meridional arc (25 Å.) and the equatorial spot (34 Å.), the two strongest spots on the original diagram. Interestingly enough, the wide-angle β pattern is maintained throughout these stages, with alterations in diffuse detail (see later). Also, certain transitional lines, clearly related to the large-spacing structure, but located on the meridian near the level of the first wide-angle layer line, remain relatively unaffected with respect to intensity and orientation. If treatment is prolonged, the specimens lose much of their strength and stiffness, and the entire pattern, at both wide and small angles, is replaced by a pair of diffuse halos (10.2 and 4.7 Å.), shown in FIGURE 4d.

The above statements indicate the stages in a frequently observed sequence of events obtained under a variety of circumstances, though sometimes with less completeness. The definitive description of the variables which influence these transitions is difficult to obtain, but it may be said that water alone at elevated temperatures can accomplish similar changes, decreased temperature slows the process, reducing agents permit faster action at a given temperature, and alcohols and other small molecules, at appropriate concentrations in aqueous solution, often facilitate development of the net diagram. It is, therefore, believed that the net diagram reflects a fundamental feature of feather keratin structure and does not require highly specific means for its disclosure. Because the application of heat and moisture apparently provides minimal conditions, the process by which the net diagram is produced may be termed, for the sake of brevity, the heat-moisture treatment.

The net diagram deserves further description. A pattern such as FIGURE 4b may be considered with respect to two groups of diffractions: (1) those on layer lines with k indices below 15, constituting the net diagram proper, as further described below; and (2) those on or near the meridian with k indices 15 and above, comprising the region of the transitional lines with relatively constant persistence of intensity (see FIGURE 4c).

The diffractions of the proper net diagram (FIGURE 5d) merit this designation for the following reasons. On the meridian are found outstanding spots with $k = 4$, 8, and 12, while off the meridian are observed spots at the points where $k = 2$, 6, 10, and, sometimes, 14, which exhibit the constant lateral Bernal coordinate, $\xi = 0.045$. Other diffractions in this

---

FIGURE 5 (*see opposite page*). Various small-angle diffraction diagrams of sea gull feather specimens: a, that of completely dry feather; b, that of a specimen photographed at atmospheric humidity; c, the result of immersing a specimen in water during irradiation; d, the net diagram proper, which is an enlargement of the center of FIGURE 4b; e, a diagram showing the return of the strong equatorial spot for a specimen similar to that of d after exposure to air for several weeks; f, an enlargement of the center of FIGURE 4c. All patterns are at the same magnification, which is about 2.3 times that of FIGURE 4. All fibers axes are vertical. To facilitate comparison with the discussion of the text, k indices are indicated for several (horizontal) layer lines of pattern a, in which also the possible h indices of the (vertical) row lines are given. The row line with $h = 1$ is the prominent, non-meridional one with $\xi \doteqdot 0.045$, spacing 34 Å., to which frequent reference is made, and on this line at the equator ($k = 0$) and at the point where $k = 4$ are the sorption-sensitive spots. Several faint spots of the net diagrams d and e are difficult to reproduce and have been indicated by dots.

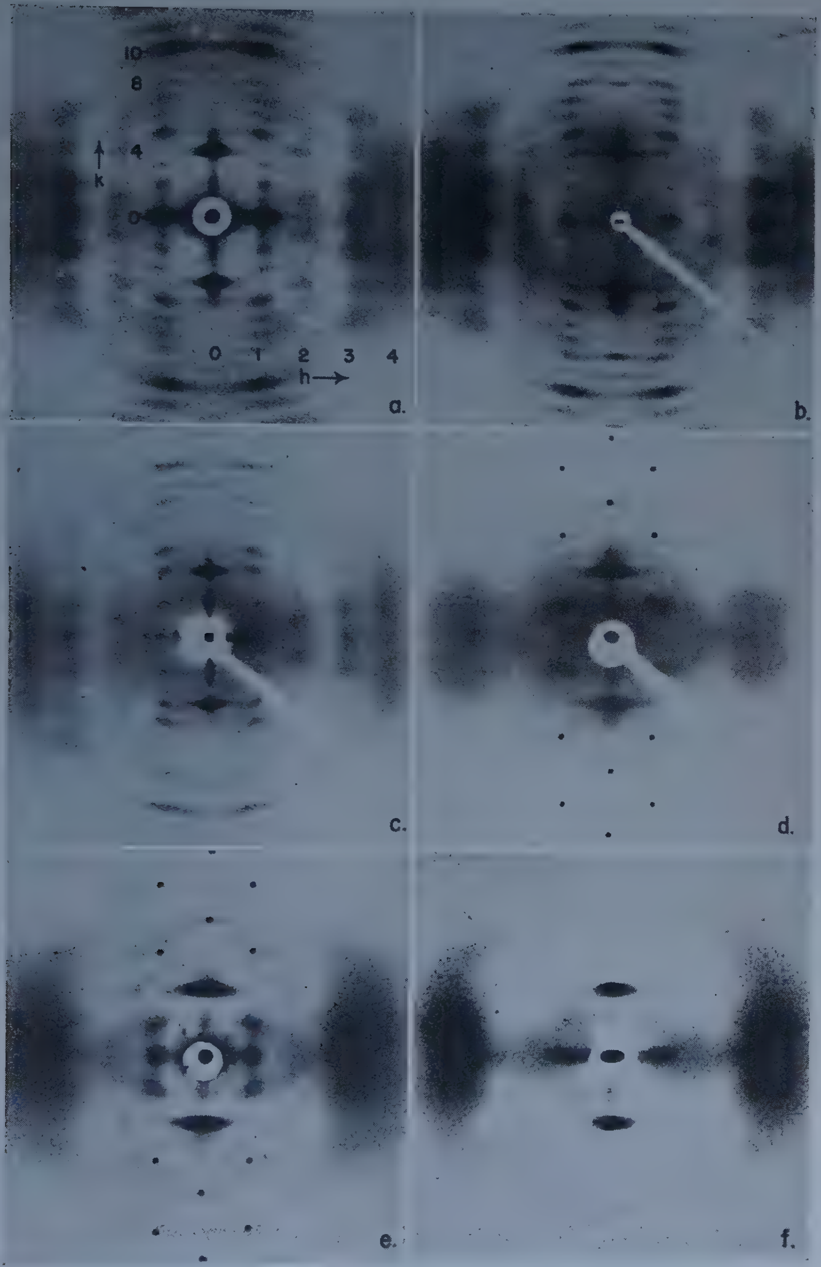


FIGURE 5 (*see facing page*).

area are either weak or considerably faded from their original intensity. The spots mentioned all observe the simple relation, that $(k/2) + h$ is even, if they are assigned appropriate h indices: 0 for the meridian and 1 for the non-meridional cases. Other diffractions consistent with this relation are sometimes observed on the more diffuse row lines further from the meridian.

It is well known from diffraction theory that this behavior clearly indicates the existence of a net, of the type whose nodes are shown by the solid circles of FIGURE 6, as a prominent part of the lattice appropriate to the fibril of feather keratin. Only the unit cell of the net is shown, and it is clear that it is twice the simplest possible face-centered net unit. This is required by the fact that the diffraction by the untreated feather specimens shows that alternate half-cells along the fibril axis must be different, even if only slightly so, to produce the 95 Å. fiber period, which is twice the simplest one for the net.

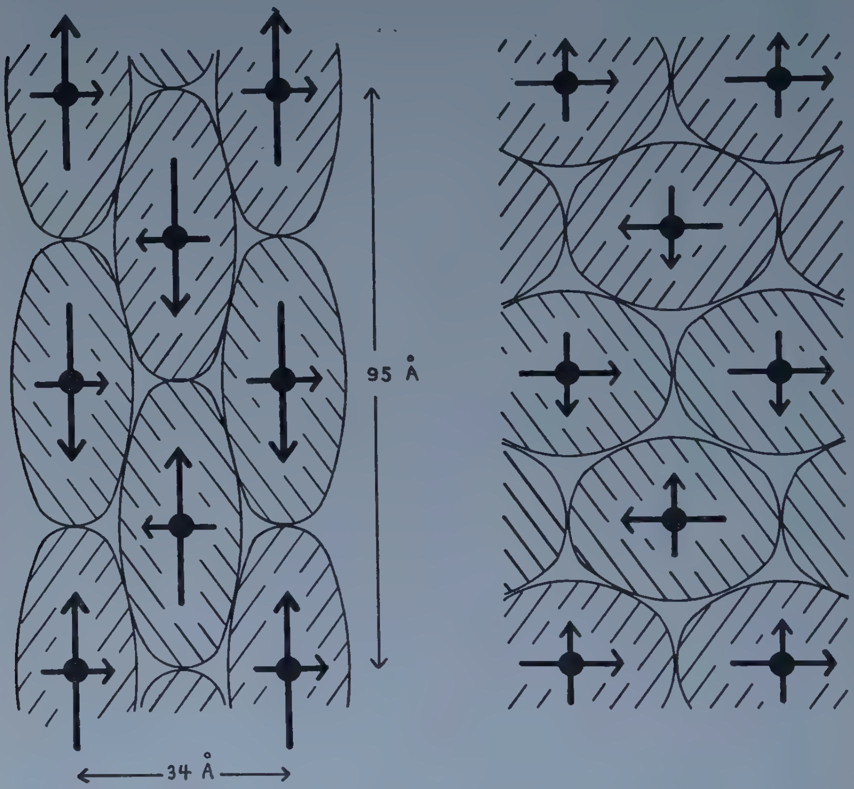


FIGURE 6. Diagrammatic representation of several stages in the present development of a model for the structure of feather-keratin fibrils. The solid circles represent the pseudo-nodes of the basic net revealed by the heat-moisture treatment. The vertical and horizontal arrows, along with the hatching, indicate a possible arrangement of orientations of identical material about each node, giving rise to the diffractions which are improper for the basic net and are observed in untreated specimens. The two figures represent extreme ways in which approximately ellipsoidal particles could be drawn about the net nodes. In each case the fibril axis is vertical.

It is believed that little alteration of the feather-keratin structure is required to make the important net-like structure of the fibrils become apparent. Rudall (1947), for example, described (confirmed in the present investigation) a sample of reptilian keratin (from a claw of *Varanus niloticus*) which normally, without treatment, yields a pattern showing unmistakable resemblance to some of the outstanding characteristics of the net diagram of feather.

The basic importance of this net is also evident in the diagram of unaltered feather specimens. This is seen at the strongest diffractions obtained near the centers of the diagrams, *i.e.*, those at the point where $k = 4$ on the meridian and at the points where $k = 0, 2$, and 4 on the prominent non-meridional row line at $\xi \doteqdot 0.045$ (see FIGURE 5a). Of these, the ones whose (hk) descriptions would be 04 and 12 are permitted for the ideal net, while the other two are not. The rather anomalous character of the latter pair is supported by their intensity behavior when various moisture concentrations are admitted to the feather specimens at room temperature.

The effect of moisture at ordinary temperature is illustrated in FIGURE 5, a through c, which shows patterns typical of three conditions: 5a, that of thoroughly dry material, obtained by desiccation over P_2O_5 or $CaCl_2$; 5b, that of material in equilibrium with 10 per cent or somewhat greater relative humidity; and 5c, that of complete immersion in water. The first two stages were observed by using the X-ray camera essentially as a desiccator with appropriate desiccants or sulfuric-acid solution added, while the last was obtained with the specimen immersed in water in a sealed thin-walled Pyrex capillary.

The photographs show that, on the prominent non-meridional row line, the spots where $k = 0$ and 4 are particularly sensitive to humidity, the former being reduced from a very high intensity level at dryness almost to zero intensity at saturation humidity, while the latter is absent at dryness but becomes more prominent as the humidity is increased. Other diffractions remain relatively unchanged in intensity, except for the 08 diffraction on the meridian, which reaches maximum intensity at intermediate humidity.

The moisture-sensitive spots are also influenced in intensity by the presence of alcohols. These substances have been introduced, for example, by subjecting feather specimens to the appropriate pure anhydrous alcohol at a temperature of 70°C. for an extended period (up to two weeks). Patterns were then taken at room temperature either with the specimen immersed in excess alcohol in a sealed capillary, or after allowing the alcohol to escape to the extent permitted under circumstances mentioned below.

The evidences of penetration of water and the alcohols are of two kinds: (1) an alteration of spot intensities and (2) a displacement of transverse spacings, most conveniently detected at the prominent non-meridional row line. While the intensity alterations produced by the alcohols often resemble those described above for water, they may be less marked. There is evidence that the intensity changes and the line displacements are complicated functions of the size and shape of the sorbed molecules and of their degree of penetration. In a rough way, as one goes higher in the series methanol, ethanol, propanol, butanol, and pentanol, one finds alcohol penetration into the structure to be progressively less evident. The sorbed molecules also become less easy to remove, requiring displacement with water and/or removal by vacuum and heat to hasten return of the pattern to normal relationships.

Since many of the diagrams used to survey these phenomena were obtained with a camera yielding rapid registration but small patterns, it is impossible to cite accurate values for the line displacements. It would appear, however, that in all of the variations described herein, the fiber period stays within the range 94 to 97 Å., while appreciably greater percentile variations occur at the prominent non-meridional row line (33 to 37 Å.), the lower values in each range corresponding roughly to specimens showing least evidence of sorption, as judged from intensity alterations.

The present diffraction indications of the sorption of alcohol molecules by keratin fibers are interesting in the light of similar conclusions reached otherwise by King (1947) and by Lundgren, Stein, Koorn, and O'Connell (1948).

The particularly intense, sorption-sensitive spot on the equator deserves special attention, since it provides strong evidence that the simple net cell of FIGURE 6 can be no more than a first approximation. Indeed, because of the persistence of this spot, the net characteristics provided by diagrams such as FIGURES 4b and 5c are not as perfectly produced when sorbable molecules, such as butanol, are absent during the heat-moisture treatment. Also, after standing in air for some time, during which time sorbed alcohol is presumably lost, the equatorial spot returns, as is shown in 5e. Otherwise, a pattern of the latter kind retains the net characteristics. Further remarks regarding the equatorial spot appear at appropriate points below.

The fact that some of the stronger diffractions, near the diagram center and appropriate for the net, do not show marked sorption sensitivity suggests that the net has a certain degree of significance, even in minimally manipulated specimens. It cannot be denied, however, that in the diagrams of such samples, there are many reasonably stable diffractions which do not immediately appear appropriate for the type of net postulated. The diffractions of the transitional group, in particular, are of this type.

The following major possibilities exist for all of the non-ideal diffractions: (1) they could provide evidence for a third lattice translation extending out of the plane of the net; or (2) they may even be legitimate *hk* diffractions of the net, arising because of the way in which material is oriented about each node. (The crystallographer will recognize that the circles of FIGURE 6 do not represent true nodes in the usual sense, but pseudo-nodes, which become nearly equivalent after partial deterioration of the structure by the heat-moisture treatment.) Either of these possibilities could apply to any non-ideal diffraction.

With regard to the first possibility, a decision could be reached if, for example, a sufficient number of different ξ coordinates could be measured to provide evidence that all diffractions do not belong to row lines appropriate to the net alone. In a number of instances, the diffractions of the prominent non-meridional row line do not appear to have identical ξ coordinates, but these variations are insufficient to provide evidence that a third translation must be added. It was hoped that the sorption of water or alcohols would enlarge such differences to make the examination of positions more definitive, but this proved not outstandingly the case, since many of the diffractions are displaced similarly. In particular, the sorption-sensitive equatorial spot appears to be displaced with the ideal-net diffractions.

While in structure analysis one usually expects a third translation to exist, this is not necessarily the case for protein fibers examined at small diffraction angles. Collagen, for example, has been found to have a structure which is basically that of the *one*-dimensional array (Bolduan and Bear, 1950). In addition, the muscle fibrils of the keratin-myosin group have exhibited diffraction evidence thus far entirely accounted for on the basis of *two*-dimensional nets (Cannon and Bear, unpublished; *cf.* also Hall, Jakus, and Schmitt, 1945). In fibrillar systems it is also to be expected that slight displacements of diffractions from ideal row-line positions can be caused by small departures from orthogonality of net axes, by small internal distortions of structure, or by limitations in fibril dimensions. One, therefore, requires marked differences in ξ coordinates to be certain of a third large translation. To date there is no unequivocal evidence that such exists.

Assuming the possible net-like character of the entire feather diagram at small angles, it becomes apparent that the obvious row lines are fairly satisfactorily indexed with $h = 0, 1, 2, 3$, and 4 (see FIGURE 5a). Of these, the two lines with $h = 3$ and 4 contain the aggregation of diffuse spots which together constitute Astbury's side-chain spacing in feather keratin. During mild heat-moisture treatment, these spots coalesce, losing their row-line structure, probably for reasons similar to those which cause transitional layer lines to persist at the meridian (see FIGURES 4b and 5d).

FIGURE 6 shows, by means of arrows and hatching, a possible way in which identical material could be oriented about each node of the net to produce diffractions other than those appropriate for the basic nodal net. It is readily demonstrable that the postulated orientations relative to the fibril axis (vertical arrows) would permit odd k diffractions at the meridian; these are observed at k values 9 and above. Diffractions with even k indices, which are also multiples of 4, should occur on the meridian; these are characteristic of the basic nodal net and are actually observed, one of them ($k = 4$) furnishing the strongest diffraction of the entire pattern. The remaining spots with even indices ($k = 2, 6, 10,$ *etc.*) are prohibited for the meridian, and none of this type are encountered at small and intermediate angles. The orientations suggested for structure transverse to the fibril axis (horizontal arrows) permit spots with odd h to occur on the equator, and, indeed, the observed diffractions whose h indices may be 1 and 3 are particularly prominent, the former being the strong sorption-sensitive diffraction frequently mentioned above. The postulated structure demands no systematic absences on non-meridional row lines, and none are found with untreated feather.

It is clear that the outstanding features of the diffraction by feather keratin at small and intermediate angles are consistent with the general net-like distribution of matter shown in FIGURE 6. This is true for normal untreated feather, as well as for the modifications produced by the heat-moisture treatment and the sorption of water and alcohols. It is now possible to examine the significance of this structure in the light of prevalent concepts of protein fibril structure.

A Model for Feather-Keratin Fibrillar Structure. While the complete meaning of the feather-keratin diffractions cannot be derived as yet, a number of interesting conclusions appear even at these early stages. First of all, whether or not there is a complete three-dimensional lattice for the large structure in evidence at small angles, the major importance of the net which is so easily demonstrated cannot be denied. Consequently, at least two, and this may be all, of the coordinate translations upon which the periodic fibrillar structure of large size is based, are now fairly accurately known. If the number of ordered axes is indeed two, the fibrils of feather keratin are in this respect like others (the muscle fibrils) of the keratin-myosin group.

Throughout discussions of the possible significance of the small-angle diffractions of protein fibers, there have seemed to be two main types of interpretation to be considered: (1) that the large spacings represent distances over which a characteristic sequence of amino-acid residues extends along polypeptide chains; or (2) that they may reflect ways in which large particulate monomers may aggregate to form the fibrillar structure. Hypotheses of essentially the first type were proposed at one time by Astbury (1943), but this general view has not encountered outstanding success (*cf.* Martin, 1946).

Astbury (1947), among others (see Corey and Wyckoff, 1936), has also considered the possibility of particulate aggregation, but suggestions of this type have remained nebulous. While perhaps not strictly fibrous, the tobacco mosaic virus gels, studied by Bernal and Fankuchen (1941) by X-ray means and recently by Wyckoff (1949) with the electron microscope, offer one of the outstanding examples of this type of organization. Even with this excellent case as a guide, and with detailed diffraction data available, it has not always been immediately apparent as to how this view was to be adapted for the true protein fibers. The large-spacing evidence is often for a size range which is quite far removed from that appropriate for deductions regarding the arrangment of individual amino acids.

It is pertinent at this point to suggest how the present results may be used to move toward a decision between the two broad possibilities for feather-keratin fibrillar structure. The diffraction results summarized above indicate that the feather-keratin fibril possesses repetitive axial structures of both large (95 Å.) and small (6.2 Å.) size. One may account for this by postulating the following type of structure, based on the net of FIGURE 6. In this figure, the outline of a possible particle or micelle has been drawn around each node (*cf.* the model for α keratin suggested by

Alexander elsewhere in this monograph*). At present, it is impossible to determine the exact ways in which the boundaries of particles are related, so that two extreme possibilities have been indicated by means of ellipsoidal molecules.

The large period corresponds to the length of two to four particles along the fibril axis, while the small period represents intra-particle structure. According to prevalent views of β-type structure, the latter period is the length of two amino-acid residues measured along extended polypeptide chains lying parallel to the fibril axis. Details of termination or folding of the chains at monomer boundaries cannot be deduced from available data.

Since the side-chain aggregation of diffractions seems related to the net system, it is probable that the side chains of the particles extend in planes paralleling the net. The wide-angle aspects of the feather pattern suggest that orientations of main chains and side chains within all particles are the same, so that, in moving from one particle to another, only 180° rotations about vertical or horizontal axes in FIGURE 6 are permitted. Because of the recognized orthogonality between side-chain and backbone directions, as mentioned in an earlier section, the latter may be expected to extend normal to the net plane within each particle.

These various suggestions as to relative orientations of intraparticle elements are cited, not as being unavoidable at the present stage, but rather as preliminary indications of how, with feather keratin, more effectually than with any other known protein fiber, it may be possible to bridge the gap between the large and small structural features. The specific proposals may be in conflict with the well-established facts (see the first section) regarding the orientations of side-chain and backbone spacings in β-keratin films and sheets. It may not be necessary, however, to regard the present fibrillar nets as belonging in the same category. Diagrams of feather-keratin specimens with higher orientation, if obtainable with small-angle diffractions intact, would be invaluable in deciding this question.

The heat-moisture treatment (perhaps also sorption) is presumed to disturb slightly, in more or less random fashion, the internal structure of the particles or their relative arrangement. When this distortion reaches the proper stage, each particle loses, in large part, its evidences of polarity. Distortion of structure transverse to the fibril axis must predominate, since the meridional diffractions are not greatly affected, even at the transitional lines of high index, as long as the fibrils maintain oriented diffraction. Additional evidence for transverse distortion is supplied at the side-chain area, where spreading occurs most markedly along layer lines, as mentioned in the preceding section. As a result of destruction of the coherence of X-ray scattering from some of the structural details, the net diagram makes its appearance to indicate the average distribution of particle centers. All particles must be identical, or nearly so, to produce the net diagram as excellently as is observed. The detailed analysis of the types of distortion reported here could be followed in a manner similar to that employed by Bunn and Garner (1947) with the polyamides, but this will not be developed at the present time.

---

* P. 653.

Correlative evidence of independent nature, regarding the size of possible monomeric units for feather keratin, is furnished by the work of Lundgren and his associates on dispersed chicken-feather keratin. In particular, it is pertinent to cite measurements (obtained by means of osmotic pressure, diffusion, and sedimentation) of the particles obtained by dispersion in hot, nearly neutral solutions containing detergent and bisulfite (Ward, High, and Lundgren, 1946). Since the dispersion was manifestly heterogeneous, it is probably only significant to say that the particles were of the approximate weight of egg albumin molecules (34,000 to 40,000). The present X-ray data do not specify the third dimension of the feather-keratin monomers, but it is easily calculated that this would be of the order of 50 to 60 Å., if Ward *et al.* were indeed dealing with monomeric units.

It does not seem profitable to go beyond this at the moment, but it is clear that the two approaches provided by chemical and diffraction methods should be capable of contributing materially to the solution of the structure of this particular keratin fiber and furnish valuable suggestions for application to others.

A word of caution may be added regarding comparisons dealing in exact relations between the sizes of monomers existing in different types of keratin fibril. Farrant, Rees, and Mercer (1947) concluded that the particles of 110 Å. diameter, which they believed to be present in fibrils from cortical cells of wool, could be compared directly with the large fibril period (95 Å.) of feather keratin. The present results show that the monomers of feather keratin more probably have lengths which are a half or a quarter of the X-ray period. Their result is in better agreement with half the α-keratin period of 198 Å., but whether this really is significant can not be determined at the moment.

Summary

A preliminary model is proposed for the structure of feather-keratin fibrils, based on results obtained largely from X-ray diffraction studies. As illustrated in FIGURE 6, the fibril is believed to be composed of large, globular particles, whose arrangement in a net-like order is consistent with many of the general features of the diffraction observed at small and intermediate angles. The net-like organization is particularly apparent after certain types of mild degradation of the feather structure. Diffraction evidence for the intrafibrillar sorption of water and alcohols is presented. A way in which intraparticle structure of small size may be related to the large-scale net is suggested.

Bibliography

ALEXANDER, P. 1949. Hydrogen bonds in the supercontraction and permanent set of wool fibers. Research (London) **2:** 246.

AMBROSE, E. J. & W. E. HANBY. 1949. Evidence of chain folding in a synthetic polypeptide and in keratin. Nature **163:** 483.

Astbury, W. T. 1940. The hydrogen bond in protein structure. Trans. Faraday Soc. **36:** 871.

Astbury, W. T. 1941. Jubilee Memorial Lecture. Proteins. Chemistry & Industry **60:** 491.

Astbury, W. T. 1943. X rays and the stoichiometry of the proteins. Advances in Enzymology **3:** 63.

Astbury, W. T. 1947. X-ray and electron microscope studies, and their cytological significance, of the recently discovered muscle proteins, tropomyosin and actin. Proc. Sixth Internat. Cong. Exptl. Cytology, Exptl. Cell Research, Supplement 1, p. 234.

Astbury, W. T. 1949. Reply to K. H. Meyer, 1949. Nature **164:** 35.

Astbury, W. T. & F. O. Bell. 1939. X-ray data on the structure of natural fibres and other bodies of high molecular weight. Tabulae Biologicae **17:** 90.

Astbury, W. T., F. O. Bell, E. Gorter, & J. van Ormondt. 1938. Optical and X-ray examination and direct measurement of built-up protein films. Nature **142:** 33.

Astbury, W. T., C. E. Dalgliesh, S. E. Darmon, & G. B. B. M. Sutherland. 1948. Structure of synthetic polypeptides. *Ibid.* **162:** 596.

Astbury, W. T., S. Dickinson, & K. Bailey. 1935. The X-ray interpretation of denaturation and the structure of the seed globulins. Biochem. J. **29:** 2351.

Astbury, W. T. & R. Lomax. 1935. An X-ray study of the hydration and denaturation of proteins. J. Chem. Soc. **1935:** 846.

Astbury, W. T. & T. C. Marwick. 1932. X-ray interpretation of the molecular structure of feather keratin. Nature **130:** 309.

Astbury, W. T., S. V. Perry, R. Reed & L. C. Spark. 1947. An electron microscope and X-ray study of actin. Biochim. et Biophys. Acta **1:** 379.

Astbury, W. T., R. Reed, & L. C. Spark. 1948. An X-ray and electron microscope study of tropomyosin. Biochem. J. **43:** 282.

Astbury, W. T. & W. A. Sisson. 1935. The configuration of the keratin molecule and its orientation in the biological cell. Proc. Roy. Soc. (London) **A150:** 533.

Bailey, K. 1948. The proteins of the muscle fiber. J. Intern. Soc. Leather Trades' Chemists **32:** 18.

Bamford, C. H., W. E. Hanby, & F. Happey. 1949. Evidence for α-protein structure of polypeptides. Nature **164:** 138.

Bear, R. S. 1943. Long X-ray diffraction spacings of the keratins. J. Am. Chem. Soc. **65:** 1784.

Bear, R. S. 1944. X-ray diffraction studies on protein fibers. II. Feather rachis, porcupine quill tip and clam muscle. *Ibid.* **66:** 2043.

Bear, R. S. 1945. Small-angle X-ray diffraction studies on muscle. *Ibid.* **67:** 1625.

Bernal, J. D. & I. Fankuchen. 1941. X-ray and crystallographic studies of plant virus preparations. J. Gen. Physiol. **25:** 111.

Bolduan, O. E. A. & R. S. Bear. 1949. Effective use of collimating apertures in small-angle X-ray diffraction cameras. J. Applied Phys. **20:** 983.

Bolduan, O. E. A. & R. S. Bear. 1950. The unidirectional nature of the large periodic structure of collagen fibrils. J. Polymer Science **5:** 159.

Brill, R. 1943. Über Beziehungen zwischen der Struktur der Polyamide und der des Seidenfibroins. Z. physik. Chem. **B53:** 61.

Brown, C. J., D. Coleman, & A. C. Farthing. 1949. Further studies in synthetic polypeptides. Nature **163:** 834.

Bull, H. B. 1945. Elasticity of keratin fibers. II. Influence of temperature. J. Am. Chem. Soc. **67:** 533.

Bull, H. B. & M. Gutmann. 1944. Elasticity of keratin fibers. *Ibid.* **66:** 1253.

Bunn, C. W. & E. V. Garner. 1947. The crystal structure of two polyamides ('nylons'). Proc. Roy. Soc. (London) **A189:** 39.

Corey, R. B. 1940. Interatomic distances in proteins and related substances. Chem. Revs. **26:** 227.

Corey, R. B. & R. W. G. Wyckoff. 1936. Long spacings in macromolecular solids. J. Biol. Chem. **14:** 407.

Elöd, E. 1941. Die Struktur der Wollfaser. Kolloid-Z. **96:** 284.

Elöd, E. & H. Zahn. 1949a. Beitrag zur Kenntnis der Superkontraktion. Melliand Textilber. **30:** 17.

Elöd, E. & H. Zahn. 1949b. Über die Voraussetzungen des Auftretens von Kautschukelastizität bei Keratinfasern. Kolloid-Z. **113:** 10.

Farrant, J. L., A. L. G. Rees, & E. H. Mercer. 1947. Structure of fibrous keratin. Nature **159:** 535.

Giroud, A. & H. Bulliard. 1930. La kératinisation de l'épiderme et des phanères. Genèse des substances soufrées de la kératine. Arch. Morphol. **29:** 7.

Hall, C. E., M. A. Jakus, & F. O. Schmitt. 1945. The structure of certain muscle fibrils as revealed by the use of electron stains. J. Applied Phys. **16:** 459.

Huggins, M. L. 1942. X-ray studies of the structure of compounds of biochemical interest. Ann. Rev. Biochem. **11:** 27.

Huggins, M. L. 1943. The structure of fibrous proteins. Chem. Revs. **32:** 195.

Jakus, M. A. & C. E. Hall. 1947. Studies of actin and myosin. J. Biol. Chem. **167:** 705.

King, G. 1947. Sorption of large molecules by keratin. Trans. Faraday Soc. **43:** 552.

Lotmar, W. & L. E. R. Picken. 1942. Über das Rontgendiagramm von gestrocknetem Muskel. Helv. Chim. Acta **25:** 538.

Lundgren, H. P., A. M. Stein, V. M. Koorn, & R. A. O'Connell. 1948. Stability of synthetic keratin fibers in alcohol-water mixtures. J. Phys. & Colloid Chem. **52:** 180.

MacArthur, I. 1943. Structure of α-keratin. Nature **152:** 38.

Marks, M. H., R. S. Bear, & C. H. Blake. 1949. X-ray diffraction evidence of collagen-type protein fibers in the *Echinodermata, Coelenterata* and *Porifera*. J. Exp. Zool. **111:** 55.

Martin, A. J. P. 1946. An investigation of a partial hydrolysate of wool. Symposium, Fibrous Proteins: 1–4. Soc. of Dyers and Colourists, Leeds, England.

Marwick, T. C. 1931. The X-ray classification of epidermal proteins. J. Textile Sci. **3:** 31.

Meyer, K. H. 1949. Classification of fibrous proteins. Nature **164:** 34.

Meyer, K. H. & C. Haselbach. 1949. Rubber-like properties of hair keratin. *Ibid.* **164:** 33.

Meyer, K. H. & H. Mark. 1928. Über den Aufbau des Seiden-Fibroins. Ber. **61:** 1932.

Nutting, G. C., M. Halwer, M. J. Copley, & F. R. Senti. 1946. Relationship between molecular configuration and tensile properties in protein fibers. Textile Research J. **16:** 599.

Palmer, J. J. & J. A. Galvin. 1943. The molecular structure of fibers made from native egg albumin. J. Am. Chem. Soc. **65:** 2187.

Rozsa, G., A. Szent-Györgyi, & R. W. G. Wyckoff. 1949. The electron microscopy of *F*-actin. Biochim. et Biophys. Acta **3:** 561.

Rudall, K. M. 1946. The structure of epidermal protein. Symposium, Fibrous Proteins: 15–23. Soc. of Dyers and Colourists, Leeds, England.

Rudall, K. M. 1947. X-ray studies of the distribution of protein chain types in the vertebrate epidermis. Biochim. et Biophys. Acta **1:** 549.

Rugo, H. J. 1949. The effect of heat and moisture on the small-angle X-ray diffraction diagram of feather keratin. Master of Science Thesis, Massachusetts Institute of Technology, Cambridge, Mass.

Schmitt, F. O., R. S. Bear, C. E. Hall, & M. A. Jakus. 1947. Electron microscope and X-ray diffraction studies of muscle structure. Ann. N. Y. Acad. Sci. **47:** 799.

Senti, F. R., M. J. Copley, & G. C. Nutting. 1945. Fibrous from globular proteins. J. Phys. Chem. **49:** 192.

Senti, F. R., C. R. Eddy, & G. C. Nutting. 1943. Conversion of globular to oriented fibrous proteins. I. By heat and mechanical working. J. Am. Chem. Soc. **65:** 2473.

Speakman, J. B. 1947. Mechano-chemical methods for use with animal fibres. J. Textile Inst. **38:** T102.

Straub, F. B. 1943. Actin. II. Studies Inst. Med. Chem. Univ. Szeged **3:** 23.

Taylor, H. S. 1941. Large molecules through atomic spectacles. Proc. Am. Phil. Soc. **85:** 1.

Ward, W. H., L. M. High, & H. P. Lundgren. 1946. Physicochemical characterization of dispersed chicken feather keratin. J. Polymer Research **1:** 22.

Waugh, D. F. 1948. Regeneration of insulin from insulin fibrils by the action of alkali. J. Am. Chem. Soc. **70:** 1850.

Whewell, C. S. & H. J. Woods. 1946. The supercontraction of animal fibres. Symposium, Fibrous Proteins: 50–57. Soc. of Dyers and Colourists, Leeds, England.

Woods, H. J. 1946. The contribution of entropy to the elastic properties of keratin, myosin, and some other high polymers. J. Colloid Sci. **1:** 407.

Woods, H. J. 1949. Reply to K. H. Meyer & C. Haselbach, 1949. Nature **164:** 34.

Wyckoff, R. W. G. 1949. The electron microscopy of macromolecular crystals. Acta Cryst. **1:** 292.

Zahn, H. 1947. Über die Struktur des α-Keratins. Z. Naturforsch. **2b:** 104.

THE EXAMINATION OF HAIR UNDER THE POLARIZING MICROSCOPE

By Stanley Marion Garn
Department of Anthropology, Harvard University, Cambridge, Mass.

Because hair is long, compared to its thickness, it is a difficult subject for thorough microscopical investigation. The fineness of the fiber necessitates relatively high magnifications, and at these magnifications the depth of focus is less than the diameter of the hair; while the narrow angular field of view limits observation to a small section of the shaft at any one time. Under these conditions, the microscope is an essentially two-dimensional viewing device, and it is necessary to alter the conditions of investigation in order to obtain simultaneous information on all three dimensions of the hair.

If the problem is approached by the use of cross sections, by the techniques of Fiala[1] or Hardy,[2] the width and breadth of the hair and the form of the cross section are visualized, but with the loss of the long dimension. Moreover, the simple cross section loses medullary detail, and it introduces a serious sampling problem; a single .02 mm. section is not an adequate sample of a 200 mm. long hair. While serial cross sections do reduce the sampling error, they still lose medullary detail and ordinarily provide no information on the orientation of the cross section, though, by the microtome candle technique of Osborne,[3] the collodion technique of Latteux,[4] or the lamination technique of Garn,[5] continuity may still be attained. Other workers, who have been interested in all three dimensions of the hair, have experimented with dark-field illumination, the differential color-illuminator of Rheinberger,[6] and with casts and replicas.[7, 8] Of the various methods noted, the familiar hair-rotator, though cumbersome, has come nearest to providing simultaneous information on all three dimensions of the hair.

The Birefringence of Hair and its Application. Hair, like nerve fibers, muscle fibers, and many natural textile fibers, is birefringent, that is, doubly-refracting. The flattened cornified cells that make up the hair are arranged parallel to the long axis of the hair. Further, the crystallites of keratin possess a similar orderly arrangement, with at least one axis parallel to the long axis of the hair. Thus, the essential birefringence of the component material is preserved by the alignment of the structural units.[9] The hair shaft is birefringent at right angles to the long axis.

Since birefringence increases with the degree of keratinization, the cortical cells are more birefringent than the medullary cells, and the more flattened cells nearer the neck of the follicle are more birefringent than the cells nearer the center of proliferation. Thus, the optical properties of the hair can be correlated with data derived from chemical analysis, differential staining, X-ray diffraction spectra, and crystallographic studies.

When a hair is subjected to investigation under ordinary transmitted light, the hair appears more or less dark against a bright background, and internal structures are visible, either if their specific absorption in the visible spectrum is sufficiently great, or if they refocus the incident light out of the plane of focus of the objective.[10] Structures differing in optical path length or in birefringence are thus not differentiated.

When a hair is examined with the polarizing microscope (or a suitably converted standard instrument), however, and the polarizers are "crossed," the background appears dark and the hair, if properly oriented, appears bright. The highly cornified structures are brightest, while the area nearest to the bulb is less bright. In the examination of skin sections, the inner and outer root sheaths and the sebaceous glands are visible without staining, since these structures also possess birefringence.[11]

Because of the phenomenon of double refraction, some of the polarized light passes through the hair at one rate, and some is transmitted at another rate. Thus, different rays of light leave the hair out of phase with respect to each other. The greater the difference between the refractive indices, or the longer the optical path length, the greater the ultimate phase difference.[12]

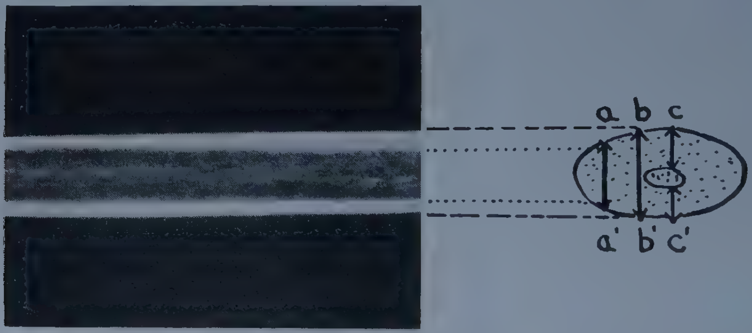


FIGURE 1. Appearance of a normally pigmented and medullated hair under the polarizing microscope. The bands indicate differences in optical path length. The effective thicknesses aa′ and cc′ are less than the thickness bb′.

Optical Staining. This difference in phase between rays of light emerging from the hair is responsible for the fact that the hair, so illuminated, appears not only bright, but also colored, due to optical interference.[12] Portions of the hair, differing in either thickness or birefringence, appear in different colors. The microscopist calls this phenomenon optical staining.

In the examination of a homogeneous, unmedullated hair shaft, these colors indicate thickness. The optical path length through the narrower edges of a hair with an oval-shaped section is less than the path length through the thicker center. The thinner edges on either side are indicated by one interference color, and the thicker center is indicated by another. The color bands so formed are continuous along areas of equal thickness, and the color bands can be employed like the contour bands on a map. When they are parallel, it may be assumed that the thickness is constant, while bands that converge or diverge indicate comparable variations in the thickness of the hair. Thus, the third dimension, that parallel to the optical axis, is as accessible as the two in the optical plane.

These color bands are especially useful when it is desired to examine lengths of hair for structural variations, nodules, twists, constrictions, or bandings.[13] Under ordinary illumination, careful scrutiny and repeated measurements with the eyepiece micrometer may be necessary to demonstrate their existence, while, under the polarizing microscope, abrupt shifts in brightness and in the color bands herald their presence.[14] This phenomenon is even more important in the examination of hairs that show narrow bandings only under the polarizing microscope. These bandings appear to be due to differences in cellular organization and orientation and are visible in no other way.

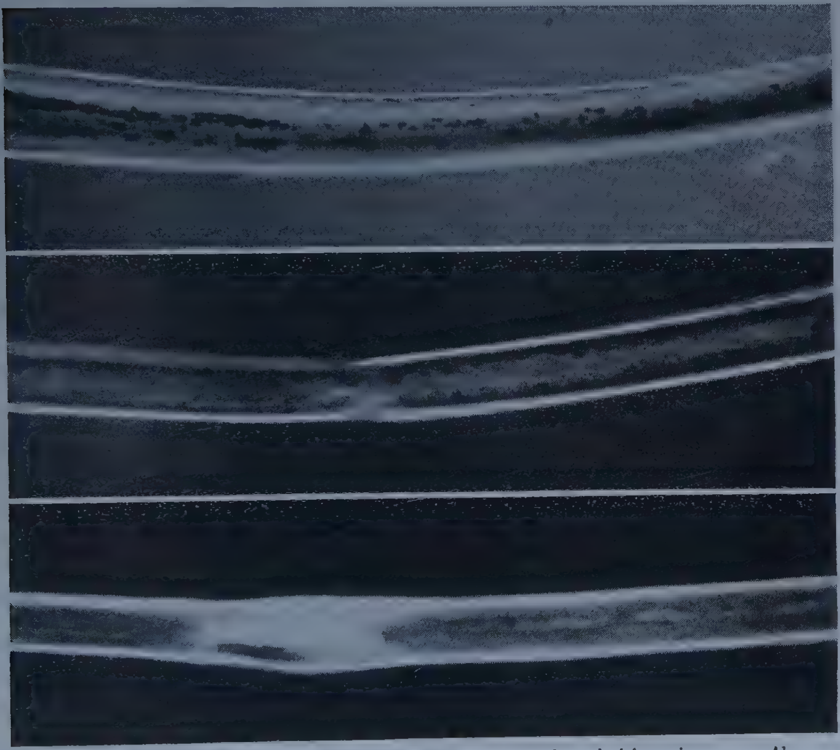


FIGURE 2. Appearance of different structural variations under the polarizing microscope. Above, a curved beard hair showing medullary pigmentation. Middle, a head hair showing a small constriction invisible under ordinary transmitted illumination. Below, a head hair showing a swelling and medullation.

Examination of the Medulla. The medulla, too, can be studied profitably with the aid of the polarizing microscope. Under ordinary illumination, a large empty medulla is a cylinder of low refractive index in a medium of higher refractive index and becomes a dispersing lens, so that the medulla appears deceptively black and may seem to be full of pigment, as microscopists have long noted.[15] Under the polarizing microscope, the medulla remains an isotropic inclusion in a birefringent substance, and the phase difference of light rays passing through the portion of the hair containing the medulla is less than the phase difference of light rays traversing surrounding parts. Hence, the medulla appears in a different color from the surrounding cortex, while pigment granules (if present) remain dark, due to their specific absorption. In this way, the pigment content of the medulla is easily differentiated from empty space.[14] This is of special utility when short sections of medullated hair are mounted in a rather thin medium, for the medulla may become filled with the medium (by capillary action) and, under ordinary illumination, may appear absent. Because the medium is not birefringent, however, the medulla is still as visible under the polarizing microscope as if it were empty. This is clearly important when hair thickness is related to the presence of a medulla or when the frequencies of different medullary types are being computed.

Optical Activity. A further property which may be investigated under the polarizing microscope is that of optical activity, as shown by rotation. This owes its name to the discovery (by Biot) that certain substances (as, for example, sugar solutions) will rotate a plane-polarized beam of light.[12] In the investigation of thin cross-sections of highly waved or helical hair, as is common in the Negro, small amounts of rotation have been observed by the author.[13] It may be emphasized that the rotatory phenomenon is observed along the long axis of the hair, while birefringence is exhibited at right angles to this axis. Since the visible rotation has come from the optical activity of thin cross sections of hair, it would seem that the amount of rotation per unit thickness of hair is higher than that usually exhibited by biologic substances, but approaches that of some liquid crystals. This finding, which requires further confirmation, represents a type of problem accessible only to the polarizing microscope.

Bibliography

1. Fiala, G. F. 1930. Am. J. Phys. Anthrop. **14:** 73–74.
2. Hardy, J. I. 1935. U. S. Dept. Agriculture. Circular 378.
3. Osborne, G. G. 1935. Textile Research **5:** 351–365.
4. Latteux, P. 1883. Manuel de Technique Microscopique (2nd ed.) Paris.
5. Garn, S. M. 1947. Science **105:** 238.
6. Anonymous. 1944. Photomicrography (14th ed.) Eastman Kodak Co. Rochester.
7. Barnes, R. B., C. J. Burton, & R. G. Scott. 1945. J. Applied Physics. **16:** 730–739.
8. Hardy, J. I. & T. M. Plitt. 1940. U. S. Gov't. Printing Office. Wildlife Circular No. 7.
9. Bunn, C. W. 1945. Chemical Crystallography. Clarendon Press. Oxford.
10. Gage, S. H. 1947. The Microscope (17th ed.) Comstock Publishing Co. Ithaca.
11. Schmidt, W. J. 1924. Die Bausteine des Tierkörpers in Polarisiertem Lichte. Fr. Cohen. Bonn.
12. Wood, R. W. 1934. Physical Optics (3rd ed.) Macmillan. New York.
13. Garn, S. M. 1946. Am. J. Phys. Anthrop. N. S. **4:** 252. (abstract)
14. Garn, S. M. 1948. Human Hair: Its Composition, Anatomy and Distribution. Doctorate Thesis. Harvard University.
15. Carpenter, W. B. 1875. The Microscope and its Revelations (5th ed.) Lindsay and Blakiston. Philadelphia.

CHANGES IN THE PHYSICAL PROPERTIES OF WOOL FIBERS PRODUCED BY BREAKING HYDROGEN BONDS WITH LITHIUM BROMIDE SOLUTIONS

By Peter Alexander* †
Research Department, Wolsey Ltd., Leicester, England

It is accepted that hydrogen bonds are the only cross links between adjacent molecular chains in cellulose and silk and, together with van der Waal's forces, are responsible for the tensile strength of these fibers.[8, 18] The X-ray diffraction diagram of nylon has been shown by Brill[8] to indicate that the NH group in one chain is opposite a CO group in the neighboring chain, enabling a powerful hydrogen bond to be formed.

It is not possible to determine the relative importance of van der Waal's forces and hydrogen bonding, but the former alone can be very powerful in highly oriented systems, as they are the only forces holding the molecules together in fibers made from polyethylene derivatives and polyesters, all of which have great tensile strength. On the other hand, the polypeptide chain in wool contains a great proportion of amino acids with large side chains[5] (50 per cent of the weight of wool is in the side chains), which prevent close packing and decrease the extent to which hydrogen bonding, and, above all, the operation of van der Waal's forces, can occur. Although the tensile strength of wool is low, compared with other textile fibers, the value would be still less were it not for the presence of disulphide cross links. Harris, Mizell, and Fourt[13] have shown the very great contribution which these bonds make to the elastic properties of wool, whose elastic modulus in water is reduced to less than one-tenth, once the disulphide bonds are ruptured.

Many of the physical properties of wool have been attributed solely to the disulphide link by Speakman[24] and his collaborators,[26] who have attempted to deduce the nature and extent of chemical changes produced during different treatments from alterations in the elastic properties, supercontraction, and permanent set bahavior. This picture was challenged by Harrisson,[14] whose experimental data, however, were not wholly satisfactory. Phillips,[20] Rudall,[21] Blackburn *et al.*,[7] and, above all, Elöd's school in Germany,[9] showed conclusively that behavior connected with the contraction of a wool fiber during a reaction (supercontraction) or the elongation remaining after a fiber has been steamed while stretched (permanent set) cannot be explained in terms of the breaking and reforming of covalent links only. All these authors have suggested that hydrogen bonding plays a part in these phenomena. Astbury[4] believes that the α fold in the peptide chain is maintained by the latter and has discussed fully the different possible hydrogen bonds which can be formed within and between molecular chains. Dorothy Jordan Lloyd,[15] in detailed investigations, attributes the swelling of protein fibers to the breaking of hydrogen bonds and believes that different reagents are capable of breaking bonds formed between different groups *e.g.*, CO . . . NH, or OH . . . OH. Elöd and Zahn[10, 29] showed that wool contracts on heating in phenol at about 80°C. and in formamide and urea at 110°C. without disulphide bond breakdown and attributed the effects produced to the breaking of hydrogen bonds.

---

* The author is grateful to Mr. H. J. Woods, Leeds University, for permission to use the results of his X-ray photographs. The author also wishes to thank Mr. K. Statham for experimental assistance and the Messrs. Wolsey, Ltd., for permission to publish this paper.

† Present address: Chester Beatty Research Institute, Royal Cancer Hospital, London S.W. 3, England.

The action of these reagents, and particularly phenol, on fibers is complex, and changes brought about by them cannot be attributed to hydrogen bond breakdown alone. Thus, for example, phenol is capable of dissolving polyethylene and polyesters in which no hydrogen bonding occurs and is incapable of dissolving silk or cellulosic fibers, which are held together almost entirely by hydrogen bonds. All these fibers swell greatly in phenol, but this is not surprising, since it is known that the latter has a greater affinity than water for sorption sites and readily replaces the latter[29] in proteins.

Solutions of cuprammonium hydroxide, as well a similar co-ordination compounds, are well known to dissolve cellulose and silk and must, therefore, be capable of breaking hydrogen bonds. These reagents were shown by Whewell and Woods[27] to produce contraction in wool, but, because of their high alkalinity, they attack the disulphide bond as well and thus cannot be used to elucidate the role of the hydrogen bond. Similarly, concentrated solutions of zinc chloride, though capable of dissolving cellulose and silk, as well as contracting wool,[14] by breaking hydrogen bonds, could not be used to study the latter, since the solutions are acid and their reactions with wool are complicated, due to main chain breakdown by peptide hydrolysis.

Strong solutions of lithium salts were shown by Meyer and Go[19] to dissolve silk and polypeptides, and we found that they are also capable of dispersing cellulosic material. Lithium salts appear unique in this respect, as no other simple neutral electrolytes show this behavior. Moreover, unlike phenol, strong solutions of lithium bromide do not increase the swelling of wool, but greatly decrease it, and this enables the effect of hydrogen bonding on the physical properties of wool to be determined without the ambiguity of interference from secondary effects.

Experimental

All measurements were made on the root ends of virgin Lincoln wool, purified by extraction with ether, alcohol, and water. The chemicals used were of analytical purity grade, with the exception of the lithium bromide, which was purified by warming aqueous solutions with wool, so as to enable any deleterious impurity to react and be removed. After this treatment, the solutions were shaken with adsorbent charcoal to remove organic matter. Analysis showed that the only impurity remaining was a small percentage of potassium, sodium, and chloride ions, none of which interfere with the reaction. The strength of all the solutions is expressed as weight of solid added to 100 cc. of water.

For the measurement of contraction, two hooks were cemented to the ends of the fiber, attached to a rod, and placed in a tube containing the test solution. The correct temperature was maintained by a water bath.

After different time intervals, the rod was withdrawn, the length measured with a steel ruler, and the fiber returned to the solution. All results on fiber length, with the exception of those shown in FIGURE 3, are the mean of at least three independent determinations.

To obtain set, the fibers were attached to an adjustable frame, placed in an 0.1 per cent borax solution (pH 9.2), stretched 30 per cent, heated under tension at 100°C. for 30 minutes, removed from the frame, and relaxed in the test solution.

The stress strain curves were determined on a simple fiber balance[24] at a constant rate of loading, which took from 5 to 15 minutes to stretch the fibers 30 per cent. The fibers were immersed in either water or the test solution while being stressed. The weakening of the fiber, expressed as the so-called "30 per cent index"[22] was determined from the ratio of the area under the stress strain curve of the fiber stressed in water to 30 per cent extension after and before treatment. It is well known that the fiber can be repeatedly stressed in water without loss in strength, so long as it is allowed to relax for 24 hours between tests.

Swelling was determined microscopically using the method of Speakman and Stott,[25] the mean of ten determinations being taken.

Results

1. *Reversible Contraction in Lithium Bromide Solutions.* FIGURE 1 shows the rate of contraction of a wool fiber when placed in a 100 per cent lithium bromide solution at different temperatures. The reaction, if carried out at below 100°C., is completely reversible, and the fiber, when placed in water, immediately returns to its normal length and regains its original physical and chemical properties unchanged (see TABLE 2).

From FIGURE 2, it is clear that only solutions stronger than 50 per cent LiBr are capable of producing a contraction and that, with concentrations greater than 75 per cent, the rate of contraction is decreased, although the final value obtained is roughly the same. In 12 hours at 22°C., a fiber will contract in 75 per cent but does not contract at that temperature in 100 per cent LiBr, even in 48 hours.

Lithium chloride reacts similarly to LiBr, but at a much slower rate (see FIGURE 1). Lithium sulphate is much less soluble, and a saturated solution (23 per cent) at 100°C. did not produce any contraction. Different wool fibers contracted to the same extent in LiBr, but the rate varied quite considerably from fiber to fiber, though the average of three results was reproducible in most cases to an accuracy of ± 10 per cent when expressed as the time necessary for half contraction. This variation cannot be attributed to differences in fiber diameter, since no correlation in the rates was obtained with fibers of widely varying diameters, nor were the rates any less variable in fibers from a carefully chosen set of the same diameter.

The fibers, on removal from LiBr, return to their original length on being placed in water, and the rate of recovery is so rapid that it is impossible to follow it with any accuracy, but it appears to be greater in hot than in cold water. The recovery occurs equally completely and rapidly in solutions varying in pH from 1 to 13, as well as in solutions saturated with potassium chloride, a 100 per cent magnesium chloride solution, or a 25 per cent LiBr solution. No recovery takes place and the fiber remains contracted when placed in a cold solution containing more than 75 per cent LiBr, and irreproducible results are obtained with 50 per cent LiBr.

2. *Irreversible Contraction in Lithium Bromide Solutions.* It was found that, after a fiber has been kept in LiBr at 100°C. for more than two hours, it no longer returns to its original length on being placed in water, but continues to contract. This effect occurs only in LiBr at 100°C. (see TABLE 1).

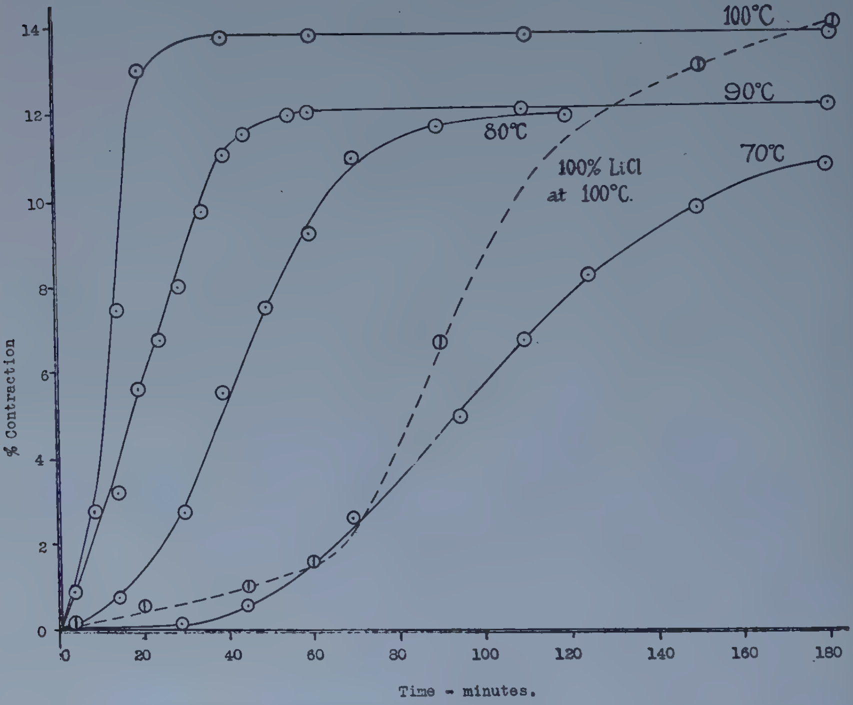


FIGURE 1. Rate of reversible contraction of wool fibers in 100 per cent LiBr at different temperatures. Also, rate of contraction in 100 per cent lithium chloride at 100°C.

The contraction of fibers, after prolonged LiBr treatment at 100°C., is very irreproducible and also depends greatly on the pH of the relaxing solution. If the latter has a pH > 8, the fiber never returns to its original length but continues to contract. If the relaxing solution is acid or neutral, then the fiber generally continues to contract, although it sometimes returns to its original length (see FIGURE 3). The cause for the occasional anomalous behavior must be found in variations from fiber to fiber, and not in the conditions of treatment, which were carefully standardized.

If the LiBr is made acid, both the amount and rate of contraction is greatly increased and the effect is completely irreversible. The presence

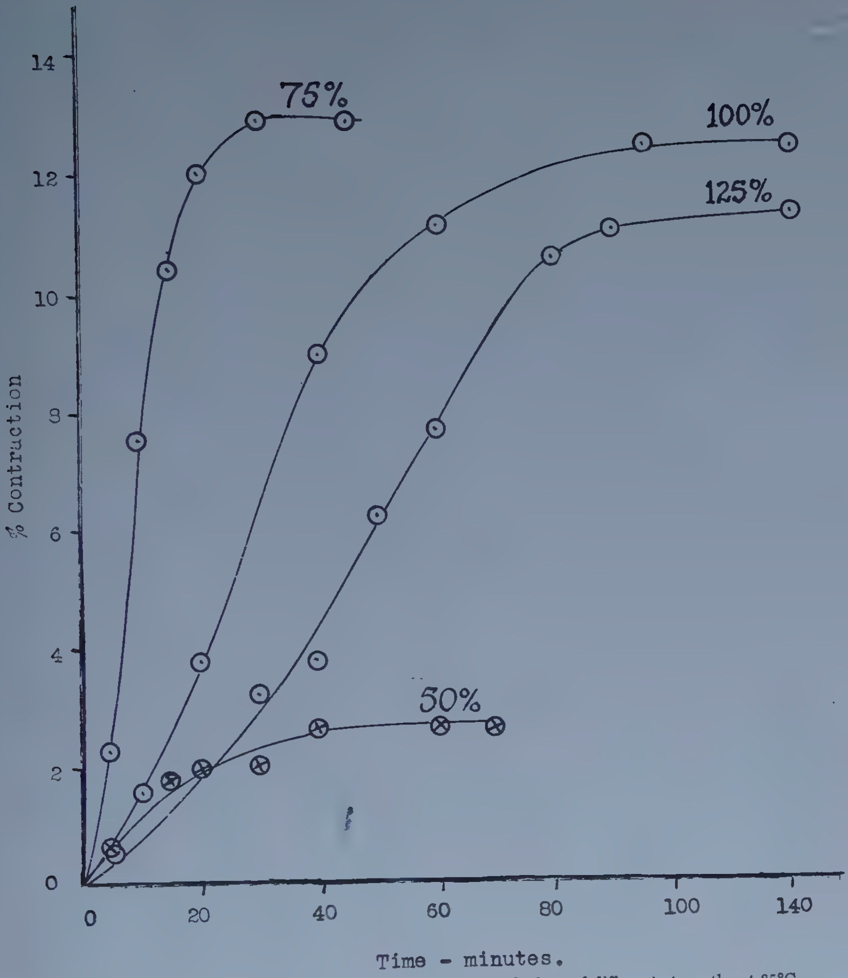


FIGURE 2. Reversible contraction of wool fibers in LiBr solutions of different strengths at 85°C.

TABLE 1

Treatment in 100% LiBr solution Temp. °C.	*Time hrs.*	*% contraction in LiBr solution*	*% contraction on being placed in water at 100°C. for 1 minute*
100	½	13.0	0.0
	1	13.0	0.5
	2	13.5	1.5
	3	13.5	16.0
90	3	12.2	1.1
80	3	11.5	0.0
	5	12.2	0.5

of 0.1 per cent borax in the LiBr does not alter its contracting properties, but the addition of N/100 NaOH increases the contraction and renders it irreversible (see FIGURE 4).

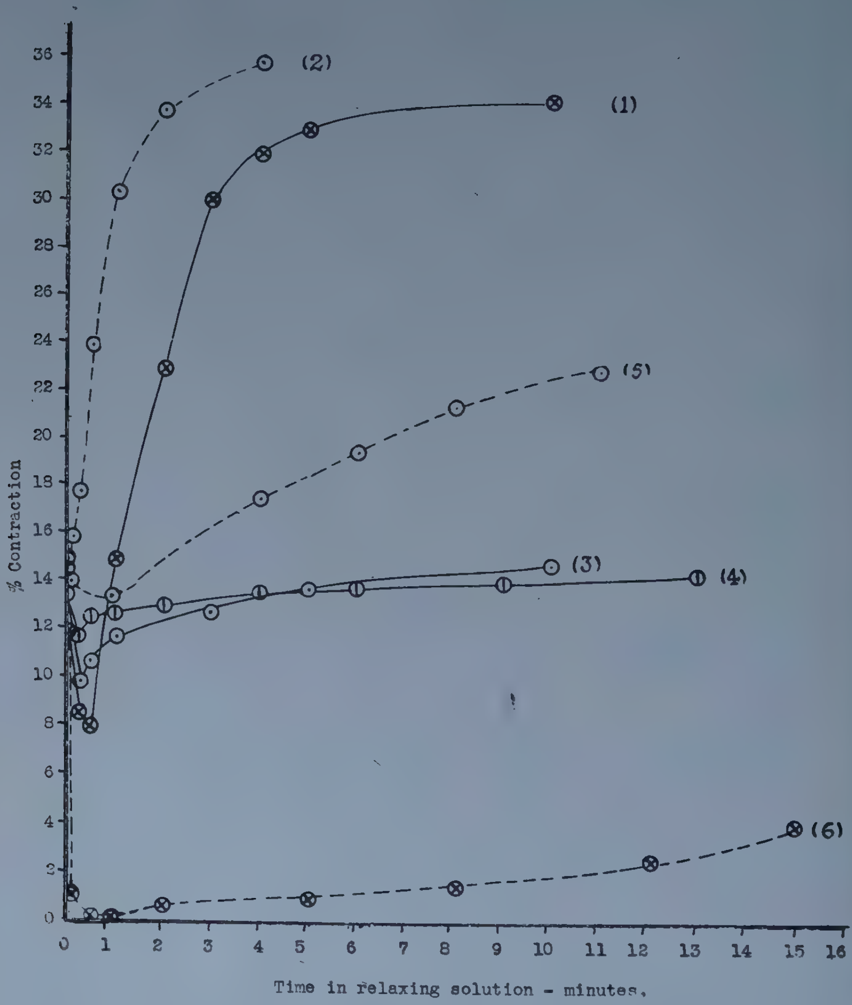


FIGURE 3. Nonreversible contraction produced by keeping fibers in 100 per cent LiBr at 100°C. for 3 hours. Change in length on placing in different solutions after LiBr treatment.
(1) Relaxed in pH 8 buffer at 25°C.
(2) Relaxed in pH 9 buffer at 100°C.
(3) Relaxed in distilled water at pH 6.3 at 100°C.
(4) Relaxed in N/10 HCl at 25°C.
(5) Relaxed in buffer at pH 7 at 100°C.
(6) Relaxed in buffer at pH 7 at 100°C. (Anomalous, typical of occasional result.)

If the fiber is given an alkaline pre-treatment, the contraction produced with 100 per cent lithium bromide is invariably irreversible, even after short periods of immersion (see TABLE 2).

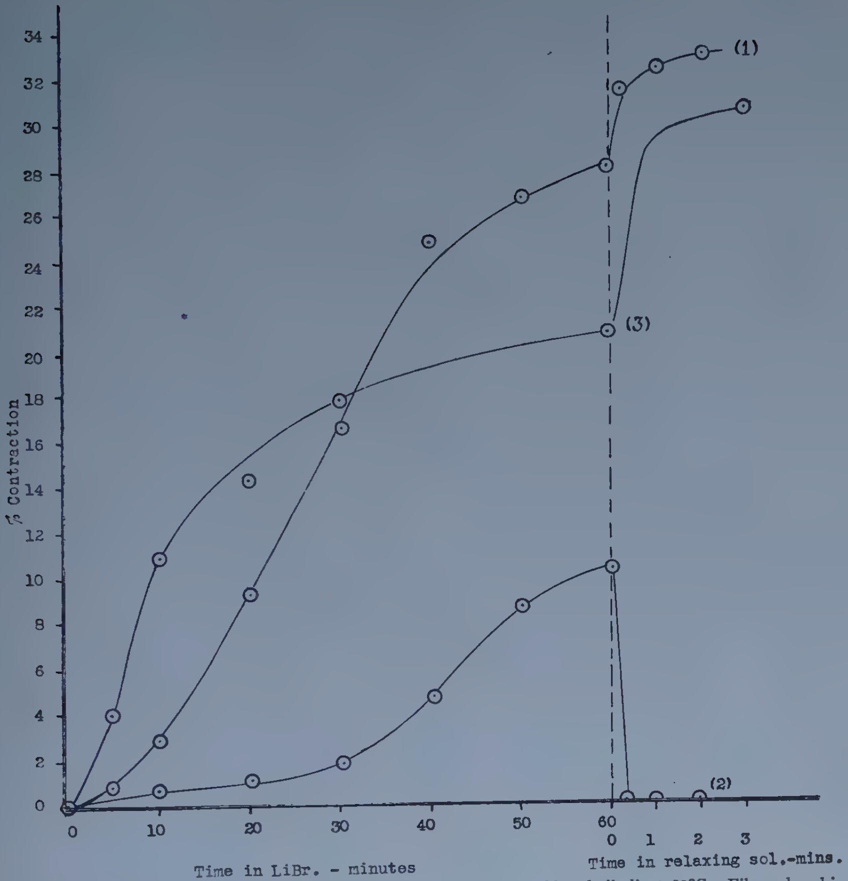


FIGURE 4. Rate of contraction in 100 per cent LiBr containing acid and alkali at 80°C. Fiber placed in distilled water at 25°C. as the relaxing solution.
(1) N/10 HCl added to LiBr.
(2) 0.1 per cent borax added to LiBr.
(3) N/100 sodium hydroxide added to LiBr.

TABLE 2

Time of pretreatment in pH 9 buffer at 100°C. minutes	*Time of reaction in 100% LiBr solution at 100°C. hrs.*	*% contraction*	
		After LiBr solution	*After 5 minutes in water at 100°C.*
None	2	14.2	−0.5
None	3	14.4	18.1
5	½	19.8	24.2
5	1	19.6	27.8
5	2	17.7	26.1
5	3	21.2	28.1
30	½	19.0	30.0
30	1	22.5	31.5
30	2	20.6	32.7
30	3	22.8	33.1

3. *Fiber Damage Produced by Lithium Bromide Solutions.* The damage suffered by a fiber during a treatment can be assessed from the decrease in work necessary to stretch it 30 per cent. The "30 per cent index" for fibers treated in 100 per cent LiBr under different conditions is recorded in TABLE 3. In each case, the fiber returned to its original length when placed in the water, in which it was restretched after a relaxation period of 24 hours.

Reversible contraction can thus be brought about without doing any damage to the fiber, but, if the period in the LiBr is extended, the fiber is weakened, even though it returns to its original length when placed in water. A permanently contracted fiber has a 30 per cent index of less than 0.1. The changes produced are not due to disulphide bond breakdown, since the cystine content of the wool is reduced only very slightly, even in treatment so severe as to produce irreversible contraction (see TABLE 4). Moreover, even after 3 hours in 100 per cent LiBr at 100°C. the wool loses less than 0.1 per cent in weight.

TABLE 3

Treatment in 100% LiBr solution Temp. °C.	*Time hrs.*	*30% index*
70	2	0.98
80	1	0.97
80	3	0.95
100	1	0.81
100	2.5	0.34

TABLE 4

3 hours in 100% LiBr solution at	*Cystine content*
80°C.	11.6
100°C.	11.7
No treatment	12.1

4. *Contraction in Solution of Phenol and Urea.* Elöd and Zahn[10, 29] discovered that wool contracts in phenol and have studied this reaction in great detail, but did not record any kinetic data or information on the reversibility of the reaction. To enable phenolic and lithium bromide solutions to be compared, the results shown in FIGURE 5 were obtained. The contractions obtained with a 10 per cent solution of phenol are small and completely reversible, except after very long periods of treatment, whereas the contraction with 50 per cent phenol is completely irreversible, and the fiber contracts still more when placed in water. In 100 per cent urea solutions at 100°C., a contraction of only 1–2 per cent took place.

5. *Contraction Produced by Breaking of the Disulphide Bond.* The powerful oxidizing agent, peracetic acid, confines its attack on wool almost entirely to the disulphide bond, which it converts to a sulphonic group.[3]

It is, therefore, particularly suitable for determining the role of disulphide bond breakdown in contraction. From TABLE 5 it is seen that 90 per cent of the cystine must be oxidized before contraction occurs without further treatment and 70 per cent before contraction occurs in a subsequent treatment with hot water.

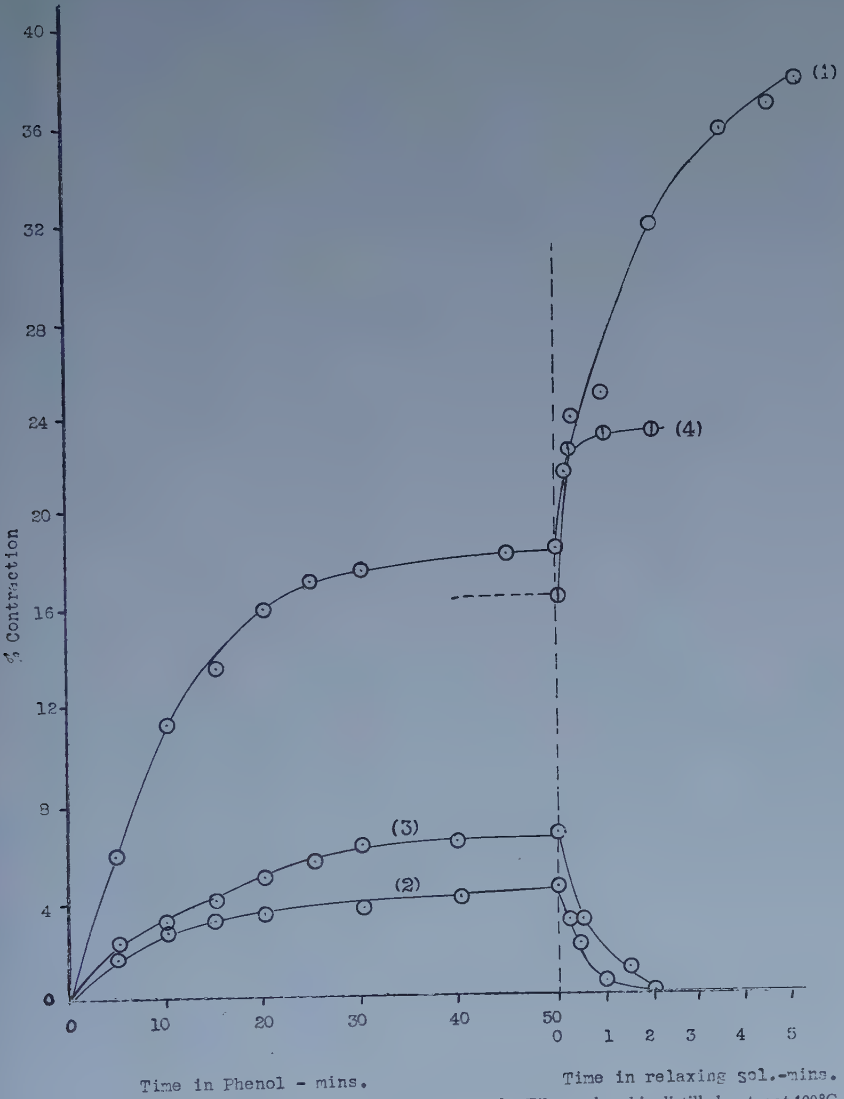


FIGURE 5. Rate of contraction in aqueous solutions of phenol. Fibers relaxed in distilled water at 100°C.
(1) 50 per cent phenol at 85°C.
(2) 10 per cent phenol at 85°C.
(3) 10 per cent phenol at 100°C.
(4) Fibers left in 10 per cent phenol at 100°C. for 7 hours and then placed into water.

Speakman[24] obtained contraction by severing the disulphide bond with a boiling solution of bisulphite. He found that when 50 per cent of the disulphide bond has been converted to lanthionine by an alkaline pretreatment, bisulphite solution no longer produces a contraction, and, from TABLE 6, it is seen that peracetic acid behaves similarly. The contraction produced with LiBr, on the other hand, is increased by the alkaline pretreatment.

6. *Effect of Lithium Bromide on Permanent Set.* When a wool fiber is stretched, and then steamed or immersed in a boiling solution of alkali, it acquires a permanent set, and, on releasing the tension, the fiber remains in an elongated form even after being placed in water at 100°C. The stability of the new structure has been attributed to the formation of new covalent cross links.[24] From FIGURE 6, it can be seen that most of the set is retained in water at 100°C. but, that in 100 per cent LiBr, the set is lost and the fiber contracts, although the contraction is slightly less than that of a fiber which had a similar alkaline pretreatment without stretching (compare TABLE 2).

TABLE 5

CONTRACTION OF WOOL OXIDIZED WITH PERACETIC ACID

Time of treatment in 1.6% peracetic acid at 22°C.	*% Cystine oxidized to cysteic acid*	*% contraction*	
		after thorough rinsing in cold water	*followed by 5 mins. in water at 95°C.*
0.5	46	0.0	1.0
1	71	0.0	22.0
25	92	20.0	41.0

TABLE 6

Treatment	*Alkali pretreatment*	*% contraction*
5% sodium metabisulphite for 1 hr. at 100°C.	None	20.3
	pH 9 buffer for 3 hrs. at 100°C.	1.0
100% LiBr for 3 hrs. at 100°C.	None	13.5
	pH 9 buffer for 3 hrs. at 100°C.	19.6
2% Peracetic acid for 24 hrs. at 20°C. followed by water at 95°C.	None	41.0
	pH 9 buffer for 3 hrs. at 100°C.	0.0

Phenol shows a behavior similar to LiBr, except that the contraction produced is distinctly less than that obtained with fibers which had not previously received a set. Fibers which had been permanently set by steaming were also released by LiBr and phenol, and, in this case, the fibers contracted to the same extent as fibers which had not been set. The set obtained by reducing fibers with compounds such as sodium thioglycollate and rebuilding with metal salts or oxidizing agents could not be released by LiBr and phenol. These treatments, however, greatly increased the tendency of the fibers to contract in LiBr, and this makes a reliable interpretation of the results impossible.

No set is obtained on heating a stretched fiber in LiBr, and, on release in the LiBr solution, the fiber contracts to the length it would have attained on heating without tension (*e.g.*, a 10 cm. fiber stretched to 13 cms. and heated for 1 hr. in 100 per cent LiBr at 100°C. immediately shortened to 8.8 cms. on release of the tension, and, on placing in water, returned to 9.9 cms.).

7. *Swelling of Fibers in Lithium Bromide.* A fiber, on being taken from water and placed into a concentrated salt solution, loses diameter within a few seconds, the water being withdrawn by an osmotic effect, and, in solutions of 50 per cent LiBr or stronger, the fiber diameter almost returns to that of the dry state (compare Sookne and Harris[22]). After standing for several hours at room temperature, or for shorter periods at elevated temperatures, the fibers begin to swell again. In solutions containing more than 50 per cent LiBr, the effect becomes complicated, since contraction sets in at the same time that the fibers begin to swell. The effect can, however, be quite clearly observed with 50 per cent LiBr solutions, which produce almost no contraction (see TABLE 7).

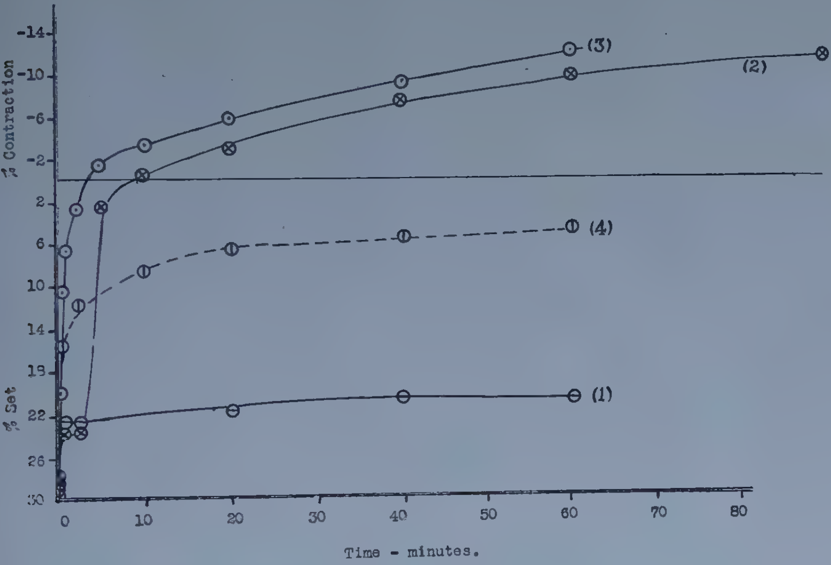


FIGURE 6. Fibers set to an elongation of approximately 30 per cent in dilute alkali. Set retained when placed in different relaxing solutions at 100°C.
(1) Distilled water.
(2) 100 per cent LiBr.
(3) 50 per cent phenol in water.
(4) 10 per cent phenol in water.

With more dilute LiBr solutions, no loss of diameter can be observed when using a microscopic measuring technique, and the fibers almost immediately swell to a diameter slightly greater than that in distilled water. Rapid loss, followed by a small increase in diameter, was observed with solutions of potassium, calcium, and magnesium chloride, but the swelling in none of these solutions was greater than that in distilled water. Aqueous solutions of phenol at room temperature immediately increase the swelling of the fibers and there is no evidence of preliminary diameter loss (see TABLE 8). The swelling in phenol may be severe without causing contraction and the two effects do not run parallel, as with LiBr.

8. *The Elasticity of a Wool Fiber in Solutions of Electrolytes and Phenol.* In TABLE 3, values are given for the reduction of work to stretch in water fibers which had previously been treated in lithium bromide solutions for different times. In this section, the elastic behavior of the fibers in the actual salt solutions will be reported. In many of the cases, the same fiber was restretched in different salt solutions after resting for 24 hours in distilled water between each extension. After repeated extension, especially in strong salt solutions, the fiber became weakened, so that its work to stretch (*i.e.*, the 30 per cent index) in water was reduced, and, when this value fell to less than 90 per cent of the original, the fiber was rejected. So as to enable comparison to be made within a series in which different fibers were used, the loads were corrected so that the extension for different fibers in water became superimposable.

TABLE 7

SWELLING OF WOOL FIBERS IN DIFFERENT AQUEOUS SOLUTIONS

Solution	*Times of immersion at room temperature*			
	30 mins.	*4 hrs.*	*12 hrs.*	*36 hrs.*
Distilled water	14.2			15.9
5% phenol	20.5			22.5
8% phenol	40.4			46.7
8% phenol + N/10 HCl	40.8			43.0
100% LiBr	1.3			0.8
75% LiBr	2.2	3.7	6.3	32.7*
50% LiBr	6.7		13.7	15.5
100% LiBr + N/10 HCl	1.5			43.2*

* Fiber has contracted.

FIGURE 7 shows the load extension curves of fibers in solutions of varying concentrations of phenol. On restretching these fibers in water, they return to their original strength, showing that the weakening with phenol is a reversible effect (compare[28]). FIGURE 8 shows a similar set of curves for fibers stretched in different concentrations of magnesium chloride and 30 per cent potassium chloride after standing for 20 hours, a sufficient time for the swelling to have attained a steady value.

FIGURE 9 shows the stress strain curves of fibers after standing for 24 hours at 22°C. in different solutions of LiBr. In 75 per cent solution, the fiber has contracted, whereas, in the 100 per cent LiBr, no swelling or contraction has taken place. In concentrations of LiBr too weak to pro-

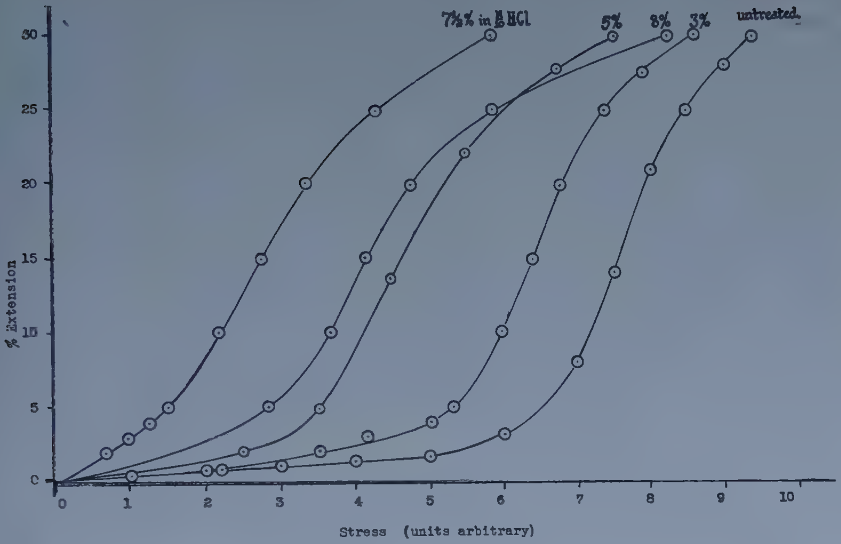


FIGURE 7. Load-extension curves of fibers stretched in aqueous solutions of phenol of different concentrations. The curve labelled "Untreated" represents the load-extension curve in water (fibers allowed to swell in phenol solution for ½ hr. before being stressed).

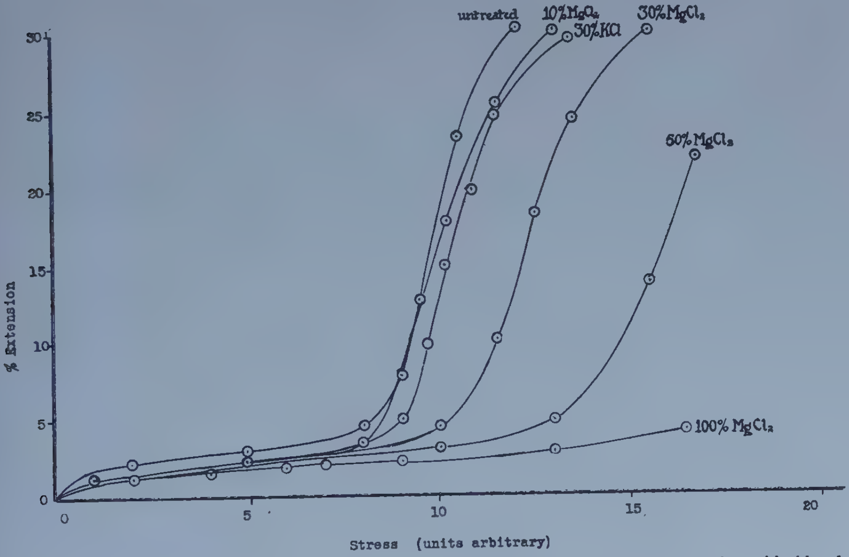


FIGURE 8. Load-extension curve of wool fibers in solutions of potassium and magnesium chloride of different strengths (per cent dry salt in water). The curve labelled "Untreated" represents the load-extension curve in water (fibers allowed to swell for 24 hrs. in the salt solutions before being stressed).

duce contraction, the fibers still behave anomalously and, unlike $MgCl_2$, $CaCl_2$, and KCl, actually lower the modulus to a value less than that in distilled water. The effect of the times of immersion in 50 per cent LiBr on the tensile properties is clearly seen from FIGURE 10. The strength of

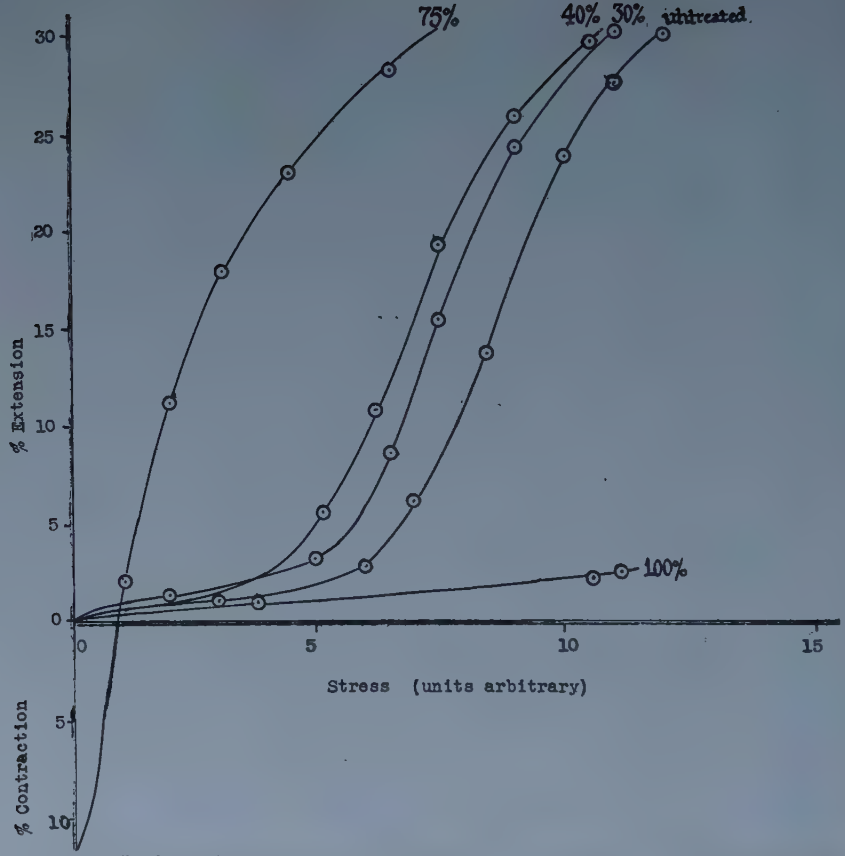


FIGURE 9. Load-extension curves of wool fibers stretched in solutions of LiBr of different strengths. The fibers were left in the solution for 24 hours to allow swelling. The fibers in 75 per cent LiBr had contracted while immersed in the cold LiBr for 24 hours. The curve labelled "Untreated" represents the load-extension curve in water.

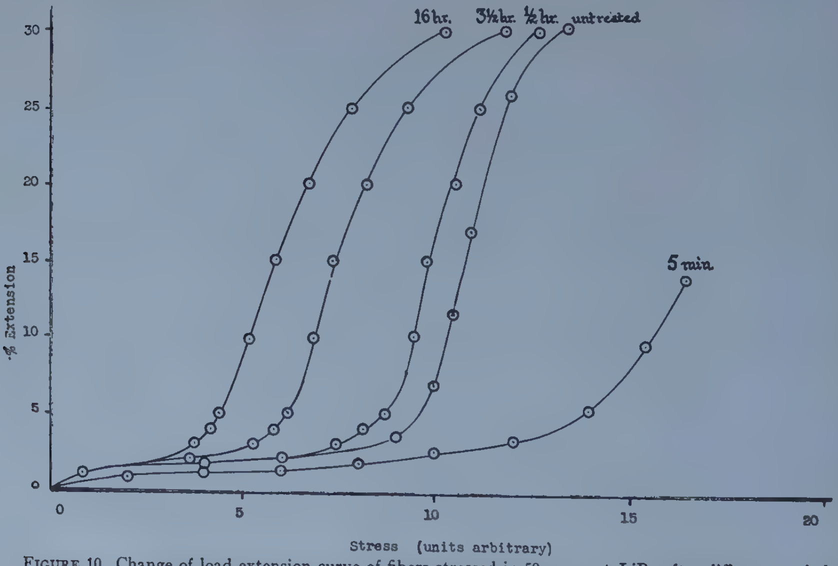


FIGURE 10. Change of load-extension curve of fibers stressed in 50 per cent LiBr after different periods of immersion at room temperature. The curve labelled "Untreated" represents the load-extension curve in water.

contracted fibers is obviously greatly reduced, although, on removal from the LiBr solution, they regain much of their strength (see TABLE 3). In solutions where no contraction has taken place, the decrease in tensile properties runs parallel with the increase in swelling.

9. *Effect of LiBr on Fibers Other than Wool.* Concentrations of LiBr in which silk or cellulosic fibers (*e.g.*, cotton and viscose rayon) do not dissolve do not produce contraction either. In strong solutions, contraction occurs before the fiber dissolves. Drawn nylon contracts in water at 100°C., and the sample used decreased in length by 5 per cent. In 100 per cent LiBr solution, the contraction was less (*i.e.*, only about 2 per cent), but the nylon filament was so gravely weakened that its tensile strength could not be determined, as it repeatedly broke during manipulation. The fact that the nylon neither dissolves nor contracts in LiBr solutions indicates the presence of strong intermolecular forces of the kind found in polyethylene and polyesters, but not present in silk or cellulose.

TABLE 8

X-RAY PATTERN OF FIBERS CONTRACTED WITH LIBR AND BY DISULPHIDE BOND BREAKDOWN WITH PERACETIC ACID (COMPARE TABLE 5)

Treatment	*% Contraction*	*X-ray Photograph*
(a) 1 hr. in 100% LiBr containing N/10 HCl rinsed with water	26	Partially disoriented β pattern
(b) 71% cystine oxidized followed by 5 mins. in water at 95°C.	22	Mixed α and β pattern; the α appears better oriented
(c) 92% cystine oxidized, rinsed in cold water only	20	Partially disoriented α pattern; no trace of β pattern
(d) As (c) followed by 5 mins. in water at 95°C.	41	Almost random β pattern

A filament of drawn Terylene (or Fiber V), composed of the polyester of ethylene glycol and terephthalic acid, as is nylon, contracted less in LiBr than in water, but, unlike nylon, its tensile strength was not affected.

10. *X-ray Diffraction Patterns of Contracted Wool.* Whewell and Woods,[27] as well as Elöd and Zahn,[11] have obtained different X-ray patterns for wool contracted by different reagents. Contraction produced with bisulphite, phenol, or steam leads to a new type of pattern showing a low degree of order, which is described either as a disoriented β or δ keratin. Fibers supercontracted with lithium bromide give a picture of this type, whereas, after contraction produced with peracetic acid, the fiber still gives an α diagram which is very similar to that of the original wool, though showing some disorder (see TABLE 8).

Thus, in a fiber contracted with LiBr, the fold of the molecular chain is completely changed, whereas only slight disordering is produced when the fiber is contracted solely by disulphide bond breakdown. The α pattern is also retained[27] in fibers contracted with dilute alkali, and this is in agreement with these findings, since this reagent confines its attack, in the initial stages, to the disulphide bond and does not break hydrogen bonds.

Discussion

1. *Mode of Action of Lithium Bromide.* Lithium is the most strongly polarizing ion and is surrounded in solution by a hydration shell, variously estimated at six to twenty molecules of water. From these experiments, it appears that, in solutions where there is insufficient water to form a complete hydration shell, the strongly positive lithium ion draws within its co-ordination orbit the negative polarized group containing hydrogen, such as NH or OH, which otherwise take part in hydrogen bonds. The co-ordination forces, in the case of hydration, are known to be very powerful, and an OH or NH group can satisfy the secondary valency bond in the same way as a water molecule. The critical concentration of lithium bromide (50 per cent), below which it does not produce contraction, corresponds to ten water molecules per lithium ion, which is a reasonable value for a hydration shell. Whether the amino or hydroxyl group forms a hydrogen bond with another component of the macromolecule, or coordinates with a metal ion, *i.e.*, whether contraction occurs, depends on the relative affinity of the two processes. The affinity of the cation increases as its water shell is decreased, since a low molecular weight polyglycine dissolves in 50 per cent LiBr, whereas a high molecular weight sample dissolves only in a 100 per cent solution.[1]

On placing the fiber in water, the lithium ion is able to complete its hydration shell, with water molecules freeing the groups in the macromolecule and enabling them to reform hydrogen bonds. Since the penetration of water into a wool fiber is very rapid, the release of the fiber is almost instantaneous. The following facts indicate that there is no direct relationship between swelling and hydrogen bond breakdown leading to contraction: (1) the fibers swell strongly in phenol in the cold and yet do not contract, (2) dilute solutions of lithium bromide (50 per cent) swell fibers as much or more than strong solutions without being able to produce contraction, and (3) formic and acetic acid, which swell wool more than phenol, do not produce contraction. Swelling can take place without primary hydrogen bond breakdown by the introduction of solvent molecules between the macromolecules, but the exact relationship between swelling and the breakdown of different hydrogen bonds has still to be elucidated. To obtain dissolution, all bonds have to be severed, and the incompletely hydrated lithium ion can do this in proteins, whereas phenol cannot.

2. *The Rate of Contraction in Lithium Bromide.* The rate curve consists of two parts, an initial lag period followed by rapid contraction. The first part is diffusion of the lithium ion into the nonswollen fiber, the hydrating water having been removed by the osmotic effect of the strong salt solution. The diffusion process has clearly a very high temperature coefficient (see FIGURE 1), although insufficient data is available for calculation. King,[17] from conductivity measurements, showed that the apparent energy

of activation for diffusion of an ion in wool increased from about 10 k.cal. to 40 k.cal. as the water content was decreased. As the lithium ion penetrates, the fiber is progressively weakened (see FIGURE 10) until, at a given point, contraction begins. The rate of contraction is greatest at the lowest effective LiBr concentration (see FIGURE 2), because the diffusion is faster at lower salt concentrations when the desiccating osmotic effect is less and the fiber retains more water.

The second stage, consisting of the relatively rapid contraction of the fibers, may be controlled by the last stages of diffusion (*e.g.*, contraction may start when the LiBr has penetrated 90 per cent of the fiber and be complete when the whole fiber has been penetrated), or it may represent the actual rate of hydrogen bond breakdown once the LiBr has diffused completely through the fiber. The fact that the speed of the second step is also greater with 75 per cent LiBr than 100 per cent LiBr (see FIGURE 2) suggested that the diffusion mechanism is operative, since the affinity of the lithium ion is increased with concentration, and the rate of reaction with hydrogen bonds would therefore not be decreased.

3. *Reversible and Irreversible Contraction.* On contraction in LiBr, the birefringence of the fibers almost completely disappears and the overall volume is increased (see TABLE 7). These facts, quite apart from the X-ray data (TABLE 8), suggest that disorientation has taken place. The micelles of peptide chains, whatever their exact shape, must be held together by hydrogen bonds, and, when these are broken, the system can assume a more random structure. A general buckling of the chains takes place, resulting in contraction and swelling, the driving force of the process being an increase in entropy. When the lithium ion is removed or rendered ineffective by having a full hydration shell, hydrogen bonds can be reformed, and the fibers are pulled from their more random buckled arrangement into alignment, resulting in a return to their original length.

Since, after a mild LiBr treatment, the fiber regains its strength completely, the molecular chains or grids can only have buckled but not moved relative to one another, and, when the hydrogen bonds are reformed, they must return exactly to their first positions. During prolonged treatments, however, the fibers, though still returning to their original length, are weakened (see TABLE 3). This suggests that a rearrangement of the chain takes place while the fiber is contracted, and that fewer or weaker hydrogen bonds are reformed on removal of the lithium ion. Contracted fibers show rubber-like elasticity, which indicates that there is very little restraint between the molecules, and movement, relative to one another, is therefore not impossible.[12]

After very prolonged treatment in LiBr, the contraction becomes irreversible, and the fibers contract still further on being placed in water. The rearrangement of the chains has proceeded to such an extent that only a very much less effective hydrogen bonding can occur. This is insufficient to pull the buckled chain back into alignment and, on removing the LiBr and reabsorbing water, the molecules coil up still more. After long treatments, the conditions are obviously critical as to whether the molecules return to an oriented form or become still more random (*i.e.*, whether the fiber returns to its original length or contracts further), and this explains the erratic results obtained after 3 hours treatment in LiBr. A pretreatment of the fiber in alkali facilitates rearrangement of the molecules in the contracted fiber (*i.e.*, irreversible contraction in LiBr is promoted), probably by breaking cross links, such as disulphide bonds. The same mechanism also explains the increased contraction, which is always irreversible in acid solutions of LiBr (see FIGURE 4), and, probably, peptide bond hydrolysis promotes increased mobility of the macromolecules in the micelle.

4. *The Micellar Structure of Wool.* Speakman[23] suggested, on the basis of indirect evidence, that the spindle cells of wool, which represent approximately 90 per cent of the total, are made up of long thin micelles, with pores of fixed diameter running between them, which are wide enough to admit ethyl, but not butyl alcohol, because the latter apparently did not swell wool. The idea of a fixed pore size had to be abandoned as a result of experiments by King,[16] who demonstrated that large molecules could penetrate the wool fiber, though slowly. Alexander, Gough, and Hudson[2] showed that the temperature coefficient for diffusion of even the small ions from aqueous solution into wool was more than twice that for diffusion in water. Diffusion in the fiber cannot, therefore, occur through free water in pores, but proceeds by hole formation, produced by deforming macromolecules in a gel-like structure.

The contraction experiments with hydrogen and disulphide bond breakers can be explained on a modification of the Speakman model, in which the micelles of polypeptide chains are linked together by disulphide bonds. Contraction produced by hydrogen bond rupture results in the buckling of the micelles, whereas disulphide bond breakdown leads to relative motion of the micelles. In the latter case, the orientation of the molecules in the micelle is not disturbed, and this explains the retention of the α pattern in a fiber contracted with peracetic acid. If the disulphide bonds are replaced by C-S-C links by an alkali pretreatment, the fiber can no longer be contracted with a disulphide bond breaker, but the action of a hydrogen bond breaker producing intermicellar contraction is not prevented (*cf.* TABLE 6). There are no fixed pores, and, for diffusion within the fiber, the micelles must be pushed apart. This accounts for the high temperature coefficient.

Not all the disulphide bonds act as cross links between the micelles and some are present within the micelles (*e.g.*, as part of the main chains or even as cross links, which, however, cannot prevent buckling). The division of the cystine in wool into fractions of widely varying reactivity suggests different arrangements. For example, although all of the cystine can be oxidized with, say, peracetic acid and chlorine solutions of a pH > 8, only 30 per cent of the total cystine can be oxidized with acid permanganate and alkaline hypochlorite solutions.[3]

No deductions can be made regarding the nature of the micelles from the results reported here, but I should like to propose that they are cylinders oriented lengthwise with respect to the fiber. The main polypeptide chains run parallel to one another in the same direction. They form the periphery of the cylinder and are held together laterally by hydrogen bonds. The nonpolar side chains point into the center of the cylinder and the polar side chains (*e.g.*,—C—S—) outwards. An arrangement of nonpolar groups directed into the inside of the micelle and polar groups outside is suggested by analogy with the soap micelle.

Such a structure is compatible with our present knowledge of the growth of the fiber. The wool protein molecules, with SH and not S-S groups, aggregate in solution into the cylindrical micelle, similar to soap molecules in solution, and leave the hair root in this form as a gel. This gel has been shown to contain free sulphydryl groups. The micelles become cross linked by the formation of disulphide bonds, the material loses its gel-like character and becomes a wool fiber.

The dry strength of the fiber must depend almost wholly on hydrogen bonds, since it is hardly affected by the oxidation of the cystine with peracetic acid. Both bonds, however, contribute to the wet strength, and breaking of either leads to a very great reduction of the modulus. True rubber-like elasticity, however, as defined by Wöhlisch[28] in terms of an increase in modulus with temperature, is only obtained after breaking of hydrogen bonds.[12] In terms of the proposed model, it is the micelles which slip, after disulphide bond breakdown, not the molecules, which are still held by hydrogen bonds and rubber-like elasticity and thus cannot arise. After rupture of the disulphide bonds, the fact that the change-over from α to β pattern occurs at much higher extensions than in untreated fibers is also explained.[12]

A disarrangement of the molecules in the micelle not sufficient to produce permanent contraction decreases the wet tensile strength (see TABLE 3) presumably by reducing the effectiveness of the hydrogen bonding, since the disulphide bonds are not attacked. Change in the stress strain curve cannot, therefore, be used (as suggested by Speakman[24]) as a measure of disulphide bond breakdown, except in special cases.

The experiments on the release of permanent set by LiBr suggest that this can also occur by two mechanisms: slippage of micelles after disulphide bond breakdown, followed by rebuilding of a covalent bond between the micelles, and rearrangement of the macromolecules within the micelles stabilized by the formation of new hydrogen bonds. In general, therefore, physical changes in the wool fiber can be brought about by intra- and intermicellar rearrangement. In the former, the X-ray picture and the density of the fiber remain almost unchanged, whereas both are altered by intermicellar reactions.

Many of the reagents studied (*e.g.*, bisulphite) at least partly attack both disulphide and hydrogen bonds. This accounts for the failure of Whewell and Woods[27] to explain the contraction produced by bisulphite, especially after pretreatments, deamination for example, only in terms of disulphide breakdown. After extensive disulphide bond rupture, the hydrogen bonding of the fiber is much more easily disturbed. This is further evidence for the presence of intramicellar disulphide links (*e.g.*, note the effect of hot water in promoting contraction in peracetic acid-treated fibers and disturbance in the X-ray pattern).

Astbury[6] has consistently advocated a unity in the behavior of muscle and keratin proteins and suggested that the mechanism of contraction in both is similar. The clear evidence presented here that reversible contraction in wool is obtained by hydrogen bond breakdown makes it tempting to postulate the same mechanism for muscular contraction.

Summary

It is shown that wool fibers contract approximately 14 per cent in hot solutions containing more than 50 g. of lithium bromide per 100 cc. of water. The contraction is reversed after the fiber is placed in water, except after prolonged treatment in the lithium bromide, when the fiber contracts still further upon being placed in water. Irreversible contraction is also produced when the salt solution is strongly alkaline or acid. Brief treatments in LiBr, sufficient to produce reversible contraction, do not damage the fiber, which regains its original elastic properties on being placed into water. After prolonged treatment, the fiber is permanently weakened, though not by attack on the disulphide bond.

The permanent set, obtained by steaming stretched fibers, is released by lithium bromide solutions. It is concluded that hydrogen bonds play an important part in the physical properties of wool fibers, and that it is wrong to attribute changes in these, resulting from a reaction only, to the breaking and rebuilding of covalent cross links.

It is suggested that solutions of lithium salts become hydrogen bond breakers when they are sufficiently concentrated to provide an insufficient amount of water of hydration for the lithium ions. The lithium ions then co-ordinate with groups in the protein molecule which normally form part of the hydrogen bridge.

Contraction can also be produced by reaction with peracetic acid. The peracetic acid confines its attack to the oxidation of the disulphide bond but this may be prevented by an alkaline pretreatment of the fiber forming C—S—C bonds. The presence of the latter, however, does not prevent contraction in solutions of lithium salts. The X-ray photos show that disorientation of the molecules is produced when a fiber is contracted in lithium bromide, but that the crystalline areas are almost unaffected by contraction resulting from disulphide bond oxidation with peracetic acid.

These facts can be explained by the assumption that the cortex of the wool fiber is made up of distinct and separate micelles, which are linked to one another by disulphide bonds. The macromolecules constituting the micelle are confined to their oriented positions by hydrogen bonds, although there must also be some intermicellar disulphide bonds. Physical changes in the fiber can be brought about by both inter- and intramicellar rearrangement.

Bibliography

1. Alexander, P., J. L. Bailey, & D. Carter. 1950. Textile Res. J. **20:** 385.
2. Alexander, P., D. Gough, & R. F. Hudson. 1949. Trans. Faraday Soc. **45:** 1109.

3. Alexander, P., R. F. Hudson, & M. Fox. 1950. Biochemical J. **46**: 27.
4. Astbury, W. T. 1940. Trans. Faraday Soc. **36**: 871.
5. Astbury, W. T. 1942. J. Chem. Soc.: 337.
6. Astbury, W. T. 1947. Proc. Roy. Soc., **134B**: 303.
7. Blackburn, S. & H. Lindley. 1948. J. Soc. Dyers & Col. **64**: 305.
8. Brill, R. 1941. Naturw. **29**: 220; 1943. Z. physik Chemie (B) **53**: 61.
9. Elöd, E., H. Nowotny, & H. Zahn. 1940. Kolloid Z. **93**: 50.
10. Elöd, E. & H. Zahn. 1944. Kolloid Z. **108**: 94; Melliand Textilber. **30**: 17.
11. Elöd, E. & H. Zahn. 1947. Melliand Textilber. **28**: 2.
12. Elöd, E. & H. Zahn. 1949. Kolloid Z. **113**: 10.
13. Harris, M., L. R. Mizell, & L. Fourt. 1942. J. Res. Nat. Bur. Stands. **29**: 73.
14. Harrisson, W. 1937. J. Text. Inst. **28**: P110.
15. Lloyd, D. J., *et al.* 1933. Trans. Faraday Soc. **29**: 554; 1936. **32**: 932; 1948. **44**: 441.
16. King, G. 1947. Trans. Faraday Soc. **43**: 552.
17. King, G. 1949. J. Colloid Science, **4**: 9.
18. Meyer, K. H. 1942. Natural and Synthetic High Polymers : p. 444. Interscience. New York.
19. Meyer, K. H. & Y. Go. 1934. Helv. Chim. Acta **17**: 1488.
    Meyer, K. H. & O. Klemin. 1940. Helv. Chim. Acta **23**: 25.
20. Phillips, H. 1946. Symp. on Fibrous Proteins (Bradford) : 39; 1947. J. Text. Inst. **38**: T122.
21. Rudall, K. M. 1946. Symp. on Fibrous Proteins. Bradford. : 15.
22. Sookne, A. M. & M. Harris. 1937. J. Res. Nat. Bur. Stands. **19**: 535.
23. Speakman, J. B. 1931. Proc. Roy. Soc. **132A**: 167.
24. Speakman, J. B. 1947. J. Textile Inst. **38**: T102 (All important earlier work in this connection is summarised in this paper.)
25. Speakman, J. B. & E. Stott. 1934. Trans. Faraday Soc. **30**: 539.
26. Stoves, J. L. 1942. Trans. Faraday Soc. **38**: 254, 261, 501.
27. Whewell, C. S. & H. J. Woods. 1946. Symp. on Fibrous Proteins (Bradford) : 50
28. Wöhlisch, E. 1942. J. pr. Chem. (N.F.) **160**: 217.
29. Zahn, H. 1947. Z. f. Naturforschung **2b**: 286.

INHERITANCE—DISTRIBUTION, PATTERN, QUALITY, AND QUANTITY

By Lorna W. Thigpen
Baptist Memorial Hospital, Memphis, Tenn.

Introduction

Differences in hair, being relatively conspicuous, have long been recognized and were among the traits early studied by geneticists. These differences include (1) those found in different races of men, *e.g.*, the types of kinky, curly, woolly, waved, and straight hair; (2) those which have been known to arise as mutations in either wild or domesticated animals, *e.g.*, kinky, curly, waved, or *rex* mutants in rodents and other domesticated animals, different coat colors and spotting patterns, and different kinds of hairless animals; and (3), more recently, those mutations which were produced by artificial means, such as irradiation, or treatment with chemicals. Due to limitations of space, only some of the representative conditions are considered here, but special emphasis is given to the hairless conditions.

Distribution. An example of an inherited difference in the distribution of hair is found in the hairs of the digits in man. Danforth[10] found that complete absence of hairs from the middle segment of the digits is due to a recessive trait.

Pattern. Kiil[23] has shown that direction of the frontal hairs in man shows three types of arrangement, determined by multiple allelomorphs. In the first of these patterns, Type I, the hairs pointed downward, with frontal convergence at the eyebrows. In Type II, there was a combination of downward and upward trends, while in Type III, the hair streamed upward from the eyebrows toward the forehead. Kiil[24] also found that Type III was present among the mongoloid patients at a state institution, suggesting some relation to the formation of the nasal bones.

In guinea pigs, there occurs a rosette formation in arrangement of the hair which, according to Wright,[47] shows a tendency towards a greater expression on the right body side. A single dominant gene R is necessary for the formation of any roughness of the familiar sort found in guinea pigs. A recessive factor m, when homozygous, gives a full rough condition in combination with R. The incompletely dominant M tends to reduce the amount of roughness. Non-genetic variation is also found. There are other modifiers of the R-Mm and R-mm patterns, *e.g.*, some strains of the genotype rr tend to develop irregularities of hair direction on the back, others on the belly, without showing the irregularities of hair pattern on the feet, which are an invariable mark of the R gene.

Nordby[37] has shown that hair whorls are inherited in swine and depend on two Mendelizing factors which must be present in either the heterozygous or homozygous condition before whorls are produced. Komai and Fukuoka[26] showed that the sinistral whorl is more common in Japanese than in white children (40 per cent against 18–20 per cent).

An unexpected variation in pattern was observed in the house mouse by crossing recessive hairless and naked mice, where the double heterozygote showed characteristic goggle eyes, a condition which is not found in mice heterozygous for the naked gene or homozygous for the recessive gene alone (Snell,[42] David[12]).

Quality and Quantity. Satin, a mutation in the rabbit (Castle and Law,[5]) produces a soft, glossy coat, highly colored. It is inherited as a simple recessive. Angora, inherited in rabbits as a recessive, produces a long, fine coat (Nachtsheim[35, 36]). There are also angora mutations in the guinea pig, cat, and goat.

Inheritance of fleece characters in sheep appear to depend on multiple factors, although there are indications that in some crosses the inheritance of the halo hairs (kemp) may be due to a single factor (Dry[18]), or to a single gene with modifying factors (Serra[41]). Nachtsheim (*loc. cit.*) suggested the possibility that the guard hairs in the rabbit might be inherited independently from the fur in view of the fact that some rabbits have poorly developed guard hairs, whereas the oppossum rabbit has many long guard hairs. In general, however, breeding experiments with sheep substantiate observations on the range and suggest a multiple factor inheritance (Burns,[2] Dry[18]). Crosses of a coarse and fine wool type, for example, will give an F_1 of intermediate texture, while the F_2, or back cross, will give intermediate types and the extremes. Serra suggests that there are at least four to six factors involved in the inheritance of fineness in wool. He thinks that about one-third of the factors involved in fleece weight are hereditary and about two-thirds are environmental. That selection has played an important role in wool production is indicated by McPhee and Spencer,[31] who state that the average fleece weight has increased from about two pounds to about eight pounds during the last century.

A simple example of the inheritance of hair weight has been shown by Trotter and Dawson[45] in crosses of French Canadians and Americans, where the heavier hair of the former is dominant over the latter.

Among rodents and other animals, there have been many mutations to kinky, curly, waved, or *rex* coats. There are six such mutants in the house mouse, four inherited as recessives, and two as dominants (Grüneberg[21, 22]). The *rex* rabbit (Nachtsheim *loc. cit.*), which has an abnormally short, soft, and plush-like coat, shows a shortening of all types of hair, including the guard hairs. Castle and Nachtsheim[6] showed that there are three types of *rex*, each a recessive mutation in a separate gene. Two of these genes, r_1 and r_2, are linked. The third gene is in a different chromosome and combines freely with the other two. All forms are similar phenotypically, and individuals homozygous for two genes (r_2 and r_3) show no greater effect than is shown in each homozygote alone. Pickard[38] described a recessive gene which caused the fine-haired *rex* rabbits to be wavy, but which did not produce waves in the coarse-haired *rex* rabbits. This is interesting, in view of the opposite condition in Karakul sheep, where Young[48, 49] found that coarse wool with very little, if any, fine underwool was essential for the highest degree of curl formation.

In humans, defects in keratinization are involved in conditions such as monilethrix and twisted hair, where hairs may break off at varying lengths. These appear to be inherited as dominant traits (Danforth,[10] Gates[20]). The woolly hair described by Mohr[34] is one of several examples of the inheritance of woolly hair in Europeans or non-negroids. In this case, the inheritance is caused by a single dominant gene. In the negro, the inheritance is less simple, but kinky hair seems to be incompletely dominant over Caucasian straight, wavy, or curly hair. Among Europeans, curly or waved hair appears to be dominant or incompletely dominant over straight hair. The mongoloid straight hair and the straight hair of the American Indian seem to be dominant over the curly hair of the Caucasians (Gates, *loc. cit.*).

Hairlessness has been observed in numerous animals to occur in varying degrees, and with different structural expressions. Landauer,[27, 28] in surveys of the literature, reported many instances, some of which were obviously inherited. More complete studies have been made on the recently occurring hairless animals, from the standpoints of genetics, development, and structure, and the interaction of factors (see David,[12–14] Grüneberg,[21, 22] Fraser[19]).

Animals in which hairless forms have been reported include the house mouse, deer mouse, mole, rat, guinea pig, rabbit, goat, swine, sheep (Serra[41]), cattle (Regan, Mead, and Gregory,[39] Wipprecht and Horlacher[46]), cat (Lethard,[29] Mellen[32]), horse, dog, and man. Differences in the normal hair of man and the other animals are striking. In comparison with the anomalies of the human skin (Cockayne[8]), those of the rodents and other forms seem relatively simple. This simplicity may be misleading, however, since minor skin defects may pass unnoticed as long as the animal retains its fur. Furthermore, the ease of breeding simplifies study of conditions in small animals, which is not true for man. No attempt is made here to go into the complicated field of human hair abnormalities.

If we examine the so-called hairless animals, we find in all cases, so far as I know, that all animals show some degree of hair formation and at least a few hairs of normal, or near normal, structure. Hence, the term hypotrichosis is more accurate. Histological studies reveal various structural changes as already suggested, and from these we may make the following classification:

(1) There may be a partial agenesis of follicles. This is found in the hairless dog, with all degrees of follicle formation from primitive anlagen to developed hairs, in rabbits (Kislovsky[25]), and in swine (David[13]). We may also add tentatively the ichthyotic mice described by Carter,[3] which are comparatively hairless and are described as differing from the other types (naked, hairless, rhino, and hypotrichosis juvenalis). These mice show a scaling and sloughing of the skin which suggests a hyperkeratosis, while the downy coat of some of these mice indicates abnormalities of the hairs. Also to be added tentatively, pending more detailed reports, is the crinkle mutant which was found following treatment with mustard gas (Auerbach and Falconer[1]). This type shows an absence of hairs on the tail and behind the ears, while the hair coat is thin, and the hairs tend to point to the mid-line of the back.

Examples of animals showing a partial agenesis of follicles as a species characteristic are the whale and the rodent *Heterocephalus* (Thigpen[43]).

(2) There may be a delay in follicle formation, as reported in cattle by Mohr and Wriedt.[33]

(3) There may be a premature keratinization of the hair follicles and sebaceous glands which may interfere with the eruption of the smaller hairs, though permitting the exit of the heavy guard hairs, as in the furless rabbit (reported by Castle,[4] and by Drapeau[15]).

(4) There may be a defective keratinization of the hairs, as in the naked house mouse (David[12]) and in the deer mouse (Clark[7]). According to Gates,[20] the shrew naturally shows a condition similar to human cases in which hairs break off because of defective keratinization.

(5) There may be a normal appearing first pelage which is lost at about the time the hairs complete growth, as seen in the recessive hairless house mouse, deer mouse, and rat described by the writer,[12] and in the rhino house mouse (Fraser[19]). These forms are characterized by abnormal hair clubs and varying degrees of hyperkeratosis and cyst formation, which leads to an increased skin thickness and, in the more pronounced form, gives a folding of the skin suggestive of the rhinoceros. This type of hairlessness is called hypotrichosis cystica.

(6) There may be an abnormality apparent only in the first hair coat of the house mouse, called hypotrichosis juvenalis by Loeffler.[30] The first coat, in extreme cases, consists of thin fuzzy, curly hairs, with the curliness sometimes confined to the tips. At about 5 weeks, the defective coat is replaced by a normal adult coat. This condition is inherited as a recessive.

Discussion. On comparing the shedding of the first hair coat in the naked, hairless, and rhino types, it is seen that the hairless animal (hr hr) sheds hair in a clean cut, definite pattern; the line of demarcation between the hairless and haired areas is sharp. On the other hand, the naked and rhino types, as well as the hairless rat and *Peromyscus* (David[12]) shed in a diffuse manner, suggestive of the normal diffuse moult in the rat (Dry[17]). Normally, the house mouse moults in a rather sharply defined pattern (Dry,[16] David[12]). A similar sharpness in the pattern loss of the hr hr mice apparently depends on the simultaneous completion of follicle growth in any given area. The diffuseness seen in the other forms apparently results from a lack of such simultaneous completion of follicle growth, or to a trapping of some of the bent hairs by the large masses of stratum corneum. In all these forms, there are alternating regenerations and losses of hairs more or less corresponding to the normal moults. These become progressively more irregular in the forms showing hypotrichosis cystica. In the naked mice, however, the heterozygotes grow new hairs at more or less normal intervals, but the defective hairs break off, or those showing a more or less amorphous structure eventually tend to work their way out of the skin. The homozygotes show extremely defective keratinization and are more irregular in hair regenerations. Hair grown later may appear more normal because the patent hair canals, opened gradually as the first hairs slowly worked their way out of the skin, now permit the passage of the poorly keratinized hairs, which appear as short corkscrews or bends. A similar condition prevails among the poorly keratinized hairs of mice obtained by crossing different kinds of hairless and waved mice, where the second generation of hairs may give the appearance of better quality than the first.

One of the conspicuous features of hairless skin, including human, may be wart-like excrescences. Among mice showing hypotrichosis cystica, these wart-like objects are due to extreme cystic development of the hair canal, the "utriculus" of earlier writers, which becomes densely packed with cornified material. On the other hand, the conspicuous wart-like protuberances on *Heterocephalus* appear to result from a specialized type of sensory hair. Most of the (mound-like) space around the hair is occupied by two large fat bodies and a large blood sinus. The skin folds in *Heterocephalus*, instead of being the result of a thickening of the skin, due to cystic proliferation of the hair follicles, as in hypotrichosis cystica, is due to localized overgrowths of the epidermis (Thigpen[43]).

Another conspicuous feature of hypotrichosis cystica is varying degrees of curliness of the regenerated hairs. This may be due to unequal cell proliferation but appears more likely to be due to growth and hardening in a curved follicle, since hair follicles which form hairs after the first coat tend to assume irregular shapes. Some curved hairs, instead of leaving the skin, may be so directed that they pierce the follicle wall, grow into the skin, and are resorbed, leaving pigmentary remains (David[12]).

The rhino and hairless genes are allelomorphs, and the effects of the former are incompletely dominant over the latter; *i.e.*, an increasing degree of abnormality results from the hr hr, the hr hr^{rh}, to the extreme $hr^{rh}hr^{rh}$ (Fraser[19]). Fraser also reports a cumulative effect involving crosses of rhino by naked. In my various studies involving crosses of hairless by naked, waved by hairless or by naked, and caracul by naked (David,[12–14] Thigpen[44]), a cumulative effect also has been found. In general, whenever any of these abnormalities of waviness or hairlessness are combined, each seems to exaggerate the effects of the other. Furthermore, as already mentioned, the gene for hairless, which normally shows no effect in a heterozygous condition, may be apparent in the goggle eyes of mice, at once heterozygous for hairless and naked (hr/+; N/+). Also, the presence of the heterozygous hr gene causes in the heterozygous naked mouse, besides a more abnormal coat, a development of the utriculus, which normally is characteristic of the homozygous hairless condition. Mice which are homozygous for hairlessness and heterozygous for naked (hr/hr; N/+), are characterized by the appearance of a woolly second coat, normally not found in either hairless or naked alone. Mice at once heterozygous for caracul and naked show the peculiarity at birth of the absence of one or both of the sensory hairs over each eye in about 90 per cent of the cases (Thigpen, unpublished[44]). This observation was made incidental to skin grafts, in which grafts appeared to be influenced by the host.

Results obtained from studies of these grafts follow briefly: Homozygous naked grafts on a non-naked host produced, in addition to the typical unerupted coiled hairs, a varying number of erupted hairs approaching normal structure. On normal hosts, these tended to be straight; on caracul hosts, they showed a tendency to curl. Naked hosts likewise seemed to influence non-naked grafts, as, *e.g.*, a caracul graft showing extremely fuzzy hairs and bent and coiled hairs typical of the naked gene. Mice, heterozygous for naked, likewise influenced their grafts, *e.g.*, a caracul graft, half apparently unaffected, half showing a fuzzy growth typical of mice heterozygous for both naked and caracul. Caracul hosts also appeared to show influence on non-caracul grafts, similar to results reported by Reed[40] for waved. Fraser (*loc. cit*) reports improved hair growths of rhino skin grafts. Actually, it is difficult to determine the cause of such improved hair growths, for, as Gersberg found in rats (Grüneberg[22]), transplantation itself appears to act as a growth stimulant. I have noticed improved growth in hairless auto-transplants; *i.e.*, more normal hairs, fewer cysts. A heterozygous naked graft which showed 4 hairs per follicle (as is usual for older normal mice), instead of the typical one or two, may possibly have shown this improvement as a result of grafting and not from substances received from its normal host. Influence from the caracul likewise is difficult to determine, since the margins of most grafts are likely to show curved hairs. On the other hand, grafts showing influence from a naked or heterozygous naked host seem more significant, since hairs of this type are distinctive and I have observed them only where this genotype is involved.

In view of (1) the localized though inconspicuous defects in keratinization in curly or waved hairs, often leading to broken tips; (2) their interaction and intensification in combination with the hairless forms; (3) the association of waved and hairless as seen in some of the *rex* rabbits (Nachtsheim[35]) and in cattle (Craft and Blizzard[9]); (4) the results of skin grafts (Thigpen, unpublished); and (5) the more or less hairless or short-haired condition which may arise as woolly hair breaks off near the surface (Gates, *l.c.*), it would seem that there is a more fundamental relationship between curly hair and hairlessness than is superficially apparent, and that, in some cases at least, they are both, but in varying degrees, an expression of one fundamental abnormality.

Summary

Some of the more typical of the inherited conditions affecting hair have been described in summary form with regard to the mode of gene expression, alone or in combination. The various types of abnormalities in so-called hairless animals are described. The different hereditary factors responsible for hairlessness and other structural changes, such as wavy hair, may show cumulative effects and also produce effects not found when only the single factors are operative.

Bibliography

1. Auerbach, C. & D. S. Falconer. 1949. A new mutant in the progeny of mice treated with nitrogen mustard. Nature (London) **163:** 678–679.
2. Burns, R. H. 1933. Wool inheritance in Hampshire-Rambouillet crossbreds. Univ. of Wyoming Agric. Exp. Sta. Bull. No. 196: 1–23.
3. Carter, T. C. & R. S. Phillips. 1949. Ichthyotic mice. Heredity **3:** 379.
4. Castle, W. E. 1933. The furless rabbit. J. Hered. **24:** 80–86.
5. Castle, W. E. & L. W. Law. 1936. Satin, a new mutation of the rabbit. J. Hered. **27:** 235–240.
6. Castle, W. E. & H. Nachtsheim. 1933. Linkage interrelations of three genes for *rex* (short) coat in the rabbit. Proc. Nat. Acad. Sci. **19:** 1006–1011.
7. Clark, F. H. 1939. Post juvenal nude in the deer-mouse. J. Hered. **30:** 213–215

8. Cockayne, E. A. 1933. Inherited Abnormalities of the Skin and Its Appendages. Oxford University Press, 1933.
9. Craft, W. A. & W. L. Blizzard. 1934. The inheritance of semi-hairlessness in cattle. J. Hered. **25:** 385–390.
10. Danforth, C. H. 1921. The distribution of hair in the digits in man. Am. J. Phys. Anthro. **4**
11. Danforth, C. H. 1925. Hair. American Medical Association.
12. David, L. T. 1932a. The external expression and comparative dermal histology of hereditary hairlessness in mammals. Z. Zellforschung u. Mikr. Anat. **14:** 617–719.
13. David, L. T. 1932b. Histology of the skin of the Mexican hairless swine (*Sus scrofa*). Am. J. Anat. **50:** 283–292.
14. David, L. T. 1937. Die gegenseitige Beeinflussung der Erbfaktoren für Haarlosigkeit und Welligkeit bei der Hausmaus (*Mus musculus*). Revue Suisse Zool. **44:** 397–400.
15. Drapeau, E. E. 1933. An anatomical study of the furless condition in rabbits. J. Morph. **54:** 365–388.
16. Dry, F. C. 1926. The coat of the mouse (*Mus musculus*). J. Genet. **16:** 287–340.
17. Dry, F. C. 1928. The agouti coloration of the mouse (*Mus musculus*) and the rat (*Mus norvegicus*). J. Genet. **20:** 131–144.
18. Dry, F. C. 1938. Genetics of live-stock production. Department of Scientific and Industrial Research. Bull. 64 (New Zealand).
19. Fraser, F. C. 1946. The expression and interaction of hereditary hypotrichosis in the mouse: histology and experimental results. Canad. J. Res. **D24:** 10–25.
20. Gates, R. R. 1946. Human Genetics, Volumes I and II. Macmillan. New York.
21. Grüneberg, H. 1943. The Genetics of the Mouse. Cambridge Univ. Press.
22. Grüneberg, H. 1947. Animal Genetics and Medicine. Paul Hoeber. New York.
23. Kiil, V. 1948. Inheritance of frontal hair directions in man. J. Hered. **39:** 206–216.
24. Kiil, V. 1948. Frontal hair direction in mentally deficient individuals. J. Hered. **39:** 281–285.
25. Kislovsky, A. D. 1928. Naked—a recessive mutation in the rabbit. J. Hered. **19:** 438–439.
26. Komai, T. & G. Fukuoka. 1934. A note on the problem of mirror-imaging in human twins. Hum. Biol. **6:** 24–32.
27. Landauer, W. 1926. Die Vererbung von Haar-und Hautmerkmalen, ausschliesslich Färbung und Zeichnung, mit Berücksichtigung von Rassendifferenzierung und Deszendenz. Z. Abstammslehre **42:** 113–226.
28. Landauer, W. 1929. Die Vererbung von Haar-und Hautmerkmalen, ausschliesslich Färbung und Zeichnung II. Z. Abstammgslehre **50:** 356–415.
29. Lethard, E. 1938. Hairless Siamese cat. J. Hered. **29:** 173–175.
30. Loeffler, L. 1934. Ueber eine neue Form von Hypotrichosis (*hypotrichosis juvenalis*) bei der Weissen Hausmaus. Z. ind. Abst. u. Vererbgsl. **67:** 209–211.
31. McPhee, H. C. & D. A. Spencer. 1936. Breeding problems with sheep. Yearbook of Agriculture **1936:** 907–929.
32. Mellen, I. M. 1939. The origin of the Mexican Hairless cat. J. Hered. **30:** 435–436.
33. Mohr, O. H. 1932. Woolly hair a dominant mutant character in man. J. Hered. **23:** 344–352.
34. Mohr, O. H. & C. Wriedt. 1928. Hairless, a new recessive lethal in cattle. J. Genet. **19:** 315–336.
35. Nachtsheim, H. 1929a. Das Rexkaninchen und seine Genetik. Zeitschr. für induktiv Abstammungs und Vererbungslehre **52:** 1–52.
36. Nachtsheim, H. 1929b. Die Entstehung der Kaninchenrassen im Lichte ihrer Genetik. Zeitschrift für Tierzüchtung und Züchtungsbiologie **14:** 53–109.
37. Nordby, J. E. 1932. Inheritance of whorls in the hair of swine. J. Hered. **23:** 397–404.
38. Pickard, J. N. 1941. Waved—a new coat type in rabbits. J. Genet. **42:** 215–222.
39. Regan, W. M., S. W. Mead, & P. W. Gregory. 1935. Inherited skin-defect in cattle. J. Hered. **26:** 357–362.
40. Reed, S. C. 1938. Determination of hair structure. I. The production of waved hair from genetically non-waved cells. J. Exp. Zool. **79:** 347–354.
41. Serra, J. A. 1948. Génétique du Mouton. Publ. Junta Pecuar. Lisboa (Ser A) No. 1.
42. Snell, G. D. 1931. Inheritance in the house mouse, the linkage relations of short-ear, hairless and naked. Genetics **16:** 42–74.

43. Thigpen, L. W. 1940. Histology of the skin of a normally hairless rodent. J. Mammalogy **21:** 449–456.
44. Thigpen, L. W. Skin grafts in mice: naked and caracul. Unpublished.
45. Trotter, M. & H. Dawson. 1934. The hair of French Canadians. Am. J. Phys. Anthrop. **18:** 443–456.
46. Wipprecht, C. & W. R. Horlacher. 1935. A lethal gene in Jersey cattle. J. Hered. **26:** 363–368.
47. Wright, S. 1949. On the genetics of hair direction in the guinea pig. I. Variability in the patterns found in combinations of the R and M loci. J. Exp. Zool. **112:** 303–323.
48. Young, C. C. 1914. Breeding of Karakul sheep. J. Hered. **5:** 170–178.
49. Young, C. C. 1922. Practical tests in Karakul sheep breeding. J. Hered. **13:** 229–236.

EFFECTS OF IONIZING RADIATION ON GROWTH AND REPLACEMENT OF HAIR*

By Friedrich Ellinger
Naval Medical Research Institute

The effect of ionizing radiation on hair represents one of the earliest observations of a biologic action of these rays.

It was in December of 1895 that Roentgen published his discovery of the X ray[1] and thus opened the field of ionizing radiation. Only three months later, in March of 1896, Professor John Daniels, at Vanderbilt University, discovered "the depilatory action of X rays," during the unsuccessful attempt to localize a foreign body in the skull.[2] In July, 1896, Marcuse, in Germany, reported the occurrence of dermatitis and alopecia, following X-ray fluoroscopy.[3] Thus, one of the fundamental biologic effects of ionizing radiations was recognized, namely, their cell destructive properties, a discovery which eventually led to the development of modern radiation therapy.

The site of action of ionizing radiation on hair is the hair papilla. This was recognized as early as 1897 by the French physicians Oudin, Barthélmy, and Darrier.[4]

Ionizing radiations affect growth and pigmentation of hair. These radiation effects may be reversible or irreversible, depending on the radiation dose applied. In principle, they are the same for man and laboratory animals.

Temporary Epilation. The reversible effect of radiation on growth of hair is called temporary epilation. It occurs in man about 8 to 10 days after exposure. The hair can be removed without pain. Later, spontaneous shading of hair takes place, and, within a period of two to three weeks, the irradiated area appears completely bald (total epilation). Usually, after 8 to 12 weeks, the growth of hair is fully restored in the irradiated area.

In man, the hair most sensitive to irradiation is that of the scalp. The hair of beard, axillae, and pubes are more resistant, and the eyelashes represent probably the most resistant type of hair in man. Similarly, Cohnen[5] found the tactile hairs of the lips of rabbits more resistant than the fur coat.

The radiation dose for temporary epilation varies considerably with type of radiation. This depends on variation in tissue absorption and quantum energy of the respective types of radiation, and other physical factors, such as size of the field of exposure, *etc.*

According to William Meyer,[6] X rays generated at 100 KV, under 0.5 mm. Al filtration, produce temporary epilation in man at 350 r/air, while at 200 KV under 2.0 mm. Cu filter, as much as 650–700 r/air may be required for this effect.

A similar dependence of the epilation effect on the type radiation was noted by Uhlman[7] in rabbits. He found the dose of temporary epilation for X rays ranging from 500 r/air at 50 KV and HVL 0.7 mm. Al, to 700 r/air at 140 KV and HVL 2.1 mm. Al. In all instances, small fields of 3 × 3 cm. were used. We[8] found the temporary epilation dose amounting to 900 r/air.[*] Gunsett and Meyer,[9] using more penetrating X rays,[†] found that 1600 r/air were necessary for a temporary epilation, which finally resulted in complete restoration of hair growth within 120 days after exposure. It thus becomes evident that, with increasing penetration power, the numerical value of r/air for temporary epilation increases too. Worning[15] determined the temporary epilation dose for guinea pigs with 1300 r/air (factors: 165 KV, 0.5 mm. Cu plus 1.0 mm. filter HVL = 0.9 mm. Cu.).

---

* The opinions are those of the author and do not necessarily reflect the official views of the Navy Department.

Let us now consider the conditions prevailing if ionizing radiations of extremely low penetration power are applied, such as the ultra-soft X rays, also known as Grenz rays. Measurements by Cipollaro and Mutscheller[10] have shown that Grenz rays generated at 10 KV are absorbed to about 50 per cent in a skin layer of 0.35 mm. and that 85 per cent are absorbed within the first 1 mm. of skin. Ebbehøj[11] carefully measured the distance of hair papillae from skin surface in rabbits and found that the majority was located within 0.2 to 0.5 mm. depth. He found a close correlation of the effect of Grenz rays with the location of hair papillae. Doses of temporary epilation have been established by Kolrep[12] for rabbits and rats with 1500 r/air (factors 10 KV, HVL 0.033 mm. Al), and by Carrié[13] with 1400–2000 r/air[‡] for mice.

Bishop[14] determined the epilation threshold for beta rays of radioactive phosphorus in rabbits with 3,500 rep. In comparison with X rays of 30 KV, it was noticed that from 3–5 times the dosage necessary to produce a given effect with these X rays is necessary for the beta rays.

Recognition of the great variability in the r/air value for temporary epilation under different physical factors of exposure to radiation indicates the extreme difficulties for an interspecies comparison of the sensitivity of hair on the base of temporary epilation. The great variety of factors used by the various investigators precludes such an attempt in most instances. It appears, however, that at least the fur coat of rabbits and guinea pigs may be considered as about 2–3 times more resistant than the average hair of man (Ellinger,[8] Klövekorn[16]).

Permanent Epilation. The occurrence of irreversible baldness or permanent epilation is entirely dependent on the radiation dose. In man, doses of 500 r/air and more of medium hard X rays (HVL of about 1.5 mm. Al) produce permanent injury to hair growth. We[8] found a ratio 1:2 for temporary to permanent epilation in rabbits. A similar ratio was also found by Allen[17] and Dahl[18] for rats and by Worning[15] for guinea pigs. However, a ratio of 1:3 was observed for dogs by Pommer and Mühling.[19] [§]

From the foregoing data, it appears that the dose for permanent epilation is remarkably uniform for different animal species, and even under quite different exposure conditions, varying between 2,000 and 3,000 r/air. The only definite exception are the Grenz rays, which, in the experience of Ebbehøj,[11] even after doses of 60,000 r/air, produced an epilation in rabbits lasting 1–2 years only.

---

[*] Factors 100 KV and 2.0 mm. Al filter HVL = 0.16 mm. Cu.

[†] Factors 200 KV and 2.0 mm. Cu filter plus 1.0 mm. Al.

[‡] AVL = 0.021 mm. Al.

[§] Ratio for rabbit 900 to 2000 r/air, factors: 100 KV, HVL 0.16 mm. Cu; ratio for rats (Allen) 1500 to 3000 r/air, factors: 200 KV, 0.1 mm. Cu plus 4.0 mm. Al filter; ratio for rats (Dahl) 800 to 2500 r/air, factors: 180 KV, 3 mm. Al filter; ratio for guinea pig 1300 to 2700 r/air, factors: 165 KV 0.5 mm. Cu plus 3.0 mm. Al filter; ratio for dogs 850 to 2400 r/air, factors: 120 KV, 1.0 mm. Al filter.

The fact that reversibility or irreversibility of the epilation effect depends largely on the radiation dose, directs the interest to exogenous and endogenous factors which may influence the effect of a given single dose of radiation. Foremost interest requires the problem of dose fractionation. Kahlstorf[20] investigated the influence of dose fractionation in rabbits and found fourfold increase in resistance. Miescher[21] irradiated the rabbit's ear with daily doses of 400 r/air and noticed an increase of the total accumulated dose for epilation in the proportion 1:3. With increase in the interval between the single exposures, this factor showed further increase. Not only the time interval, but also the size of the daily dose, influences the epilation effect. In a later study, Miescher[22] demonstrated that equal effect was produced with once-weekly exposures of 1000 r with an accumulated dose of 6000 r, or twice-weekly 600 r with a total dose of 7200 r, and, finally, six times-weekly 200 r when the total accumulated dose of 9000 r was reached. Faber[23] undertook a detailed study of the influence of dose fractionation on the epilation effect in guinea pigs, and found equal effect with 30 × 150 r, or a total 4,500 r in 30 days, and 15 × 225 r, or a total of 3,375 r in 15 days.

Among the endogenous factors, the great variation in the response of individual animals of one species should be mentioned. Mottram and Gray[24] demonstrated that the dose for permanent epilation on the tail of mice may vary between 2500 and 7000 r for X rays of 190 KV and for gamma rays between 4000 and 8000 r. Variations of similar magnitude have been observed by Worning[15] in guinea pigs. Worning concludes that the degree of the local effect of X rays on the hair is partly dependent on a universal effect. Besides the well-known genetic differences, Uhlmann[7] has indicated the influence of nutrition on the epilatory effect in rabbits. Acidifying food (oats) increases the sensitivity of this animal species. Another factor of importance is the hormones. Thyroid hormone, which in our experience sensitizes the erythemic response of rabbit and human skin,[25] leaves, however, the epilation dose uninfluenced in rabbits.[8] Hetzar,[26] however, observed regeneration of hair in rat skin exposed to doses usually resulting in permanent epilation under the influence of thyroid hormone.

Growth after Temporary Epilation. The appearance of the hair growing after temporary epilation is of equal importance in medicine and biology. As early as 1906, Imlet and Marquès[27] described a pigment anomaly of hair, namely the occurrence of dark hair in the white beard of a physician who had used X rays for quite some time without any protection. Growth of hyperpigmented hair in blond patients who had developed baldness in the course of X-ray treatment for malignancies had been described by Falke,[28] Rostock,[29] and Liborius.[30] Liborius also reported unusual curliness of regrowing hair. Regrowth of scanty and dry hair in five Negro children was observed by Hazen.[31] Dark hair regrowing in the scalp of aged patients whose hair had already turned white has been reported by Palumbo,[32] following radium therapy, and by Weiss,[33] after X-ray therapy.

In addition to these pigment anomalies in the hair of man, Hance and Murphy[34] described for the first time, in 1926, formation of white hair in colored mice after X ray irradiation. This pigment anomaly has not been described in human beings. Although primarily observed in mice, it is not specific for this species. Danneel and Lubenow[35] observed similar changes in rabbits after exposure to X rays of 70 KV, and Ebbehøj[11] studied this phenomenon in detail in rabbits after exposure to Grenz rays. Cameron[36] and Dobrovolskaja-Zavadskaja[37] reported that the decoloration of the fur of dark mice was not limited to the exposed field. Hance,[38] in a later paper, maintained that formation of white hair occurred in heterozygote animals, while in homozygotes darker hair developed.

Danneel and Lubenow[35] suggested a radiation effect on the mitochondria as a possible mechanism for this remarkable pigment anomaly. They were able to demonstrate that this effect persisted in five subsequent generations of hair regrowing in the irradiated area after repeated mechanical epilation. Murray,[39] as well as Ebbehøj,[15] demonstrated the dose relationship of this graying effect. Chase[40] demonstrated the influence of dose rates varying from 5 r/min. to 600 r/min. He observed that the higher the dose rate, the greater the response, as regards graying. Like epilation, this pigment anomaly varies with the physical properties of the radiation employed. Chase, Quastler, and Skaggs[41] compared the effect of 200 KV and 20 million volt X rays. They found "the effectiveness of high energy rays with regard to graying of the coat of mice 70 per cent of the effectiveness of low energy rays."

Finally, Chase[42] devoted a detailed study to the analysis of the problem of X ray-induced graying of hair in mice. From his studies, it appears that radiation effect on hair growth and graying of hair are two independent phenomena, but both evidently are depending on the age of hair follicles. Inactive (telogen) follicles, when irradiated, give, in succeeding hair generations, the maximum graying response. Active follicles (so-called anagen follicles) lose hair already present, and the succeeding generations of hair are less gray. Chase also confirmed the persistence of the graying effect in mice, while he was not able to confirm the observation of Hance[38] as to the significance of heterozygoty for this phenomenon and the spreading of the effect reported by Cameron.[36]

In closing this review of the effects of ionizing radiation on hair, their multi-varied significance in biology and medicine should be pointed out. These effects have been found to be a valuable tool not only for the elucidation of such biologic processes as the pigment formation, but also for the study of problems in fundamental radiation biology such as the "quantum hit theory" (Worning[15]). In medicine, the observation of radiation effects on hair has not only initiated the development of modern radiation therapy, but, most recently, it has become an important indicator for the degree of radiation injury sustained in atomic warfare, which will be discussed in detail by Dr. Liebow. Finally, radiation effects on hair have been used successfully in experimental radiation therapy for the evaluation of surface effects of new radiation sources, as demonstrated by Chase, Quastler, and Skaggs.[41]

Bibliography

1. Röentgen, W. K. 1895. Eine neue Art von Strahlen. Sitzungsber. d. Würzburger Physik. med. Gesellschaft.
2. Daniel, J. 1896. The depilatory action of X rays. N. Y. Med. Rec. **49:** 595.
3. Marcuse, W. 1896. Dermatitis und Alopecie nach Durchleuchtungsversuchen mit Röntgenstrahlen. Dtsch. Med. Wochschr. **22:** 481.
4. Oudin, Barthélmy, & Darrier. 1897. Accidents cutanés causés par les rayons X. Congrés de Moscou. Gazette des hôp. civ. et milit. **16:** I.
5. Cohnen, F. 1931. Ueber histologische Veränderungen bei Epilationsbebestrahlungen von Kaninchenhaut. Thesis, Bonn.
6. Meyer, W. 1935. The relation of air ionization to radiation absorbed and the effect on body tissues. Radiology **25:** 198.
7. Uhlmann, E. 1933. Experimentelle Untersuchungen über den Einfluss der Ernährung auf die Strahlenempfindlichkeit tierischen Gewebes. Strahlentherapie, **46:** 550.
8. Ellinger, F. 1932. Untersuchungen über die Hautwirkungen von Röntgenstrahlen am Kaninchen. I. Die Wirkungen einer mittelharten Strahlung und ihre Beeinflussung durch Thyroxin. Strahlentherapie **43:** 357.
9. Gunsett, A & I. Meyer. 1931. Variation de la radiosensibilité du tissu cutane humain et animal et de la glande genitale male du lapin et fonction de l'espacement du rayonnement. C. r. Soc. Biol. Paris. **107:** 58.
10. Cipollaro, A. C. & A. Mutscheller. 1940. Absorption of roentgen rays by the skin. Arch. Dermatol. **41:** 87.
11. Ebbehøj, E. 1937. Untersuchungen über ultraweiche Röntgenstrahlen, ihre Tiefendosiskurven und das Verhältnis zwischen ihrer biologischen Wirkung. Thesis Kopenhagen. Nyt Nordisk Forlag. Kopenhagen.
12. Kolrep, M. 1932. Ueber Gefahren der Ueberdosierung mit Grenzstrahlen beim wachsenden Organismus. Ztschr. f. Kinderheilk. **53:** 121.
13. Carrie, C. 1933. Untersuchungen über Sensibilisierung gegen Grenzstrahlen. Strahlentherapie **46:** 697.
14. Bishop, F. W. Comparison of X and beta radiation effects in rabbits. MDDC 203.
15. Worning, B. 1937. A quantitative roentgen-biological method, with the back hairs of guinea pigs as experimental objects. Thesis, Copenhagen.
16. Klövekorn, G. H. 1934. Die Epilationsbestrahlung der Kaninchenhaut. Strahlentherapie **51:** 689.
17. Allen, F. M. 1939. Local asphyxia and roentgen irradiation of transplantable rat tumors. Am. J. Roentg. **42:** 745.
18. Dahl, B. 1937. Tierexperimentelle Untersuchungen über die epilatorische Wirkung der Roentgenstrahlen in ihrer Beziehung zur Roentgendermatitis, sowie den Einfluss der Bestrahlungstechnik auf diese Wirkung. Strahlentherapie **58:** 336.
19. Pommer, A. & W. Mühling. 1931. Die Röntgenepilations- und Erythemdosis der haut bei Hunden. Arch. f. Tierheilk. **64:** 35, 60.
20. Kahlstorf, A. 1930. Untersuchungen über die Hauttoleranz bei protrahiertfraktionierter Roentgenbestrahlung, Strahlentherapie **38:** 499.
21. Miescher, G. 1930. Karzinomtherapie mit superponierten (verzettelten) Roentgenbestrahlungen. Strahlentherapie **36:** 434.
22. Miescher, G. 1934. Tierexperimentelle Untersuchungen über den Einfluss der Fraktionierung auf den Späteffekt. Acta Radiologica (Stockholm) **16:** 25.
23. Faber, B. 1939. Experimentelle Untersuchungen über die Bedeutung der Protrahierung und Fraktionierung. Acta Radiologica (Stockholm) **20:** 170.
24. Mottram, J. C. & L. H. Gray. 1940. The relative response of the skin of mice to X-radiation and gamma radiation. Brit. J. Radiol. **13:** 31.
25. Ellinger, F. 1945. Endocrine influences on the radiosensitivity of the skin and the thyroid. Arch. Dermatol. **51:** 198.
26. Hetzar, W. 1938. Die Röntgenempfindlichkeit der Haut, insbesondere in Abhängigkeit von innersekretorischen Einflüssen. (Experimentelle Untersuchungen an der Rattenhaut.) Fortschr. Röntgenstrahlen. **58:** 291.
27. Imlert, Marquès. 1906 C. r. hébdom. Séanc. Acad. Sci. 143.
28. Felke. 1926. Abnorme Haarregeneration nach Rontgenepilation. Münchner Med. Wochschr. **73:** 429.
29. Rostock, P. 1928. Seltene Haarpigmentierung nach Rontgenbestrahlung. Strahlentherapie **27:** 800.
30. Liborius, E. 1933. Ueber Lockenbildung und andere Haarveränderungen im Gefolge der Roentgenepilation. Thesis, Bonn.

31. Hazen, H. H. 1947. Results of repeated epilation with roentgen rays in tinea tonsurans. Arch. Dermatol. **56:** 539.
32. Palumbo, V. 1936. Ueber einige durch radioaktive Bestrahlungen hervorgerufene biologische Reaktionen. Strahlentherapie **56:** 318.
33. Weiss, K. 1941. Zur Frage der Hyperpigmentation des Haares nach temporärer Epilation. Strahlentherapie **69:** 340.
34. Hance, R. T. & J. B. Murphy. 1926. Studies on X ray effects XV. The prevention of pigment formation in the hair follicles of colored mice with high voltage X ray. J. exp. Med. **44:** 339.
35. Danneel, R. & E. Lubenow. 1936. Zur Physiologie der Kälteschwarzung beim Russenkaninchen. II. Mitt. Biol. Ztbl. **56:** 572.
36. Cameron, J. A. 1938. Hair color changes in mice as indicators of the spread of X ray effects. Genetics **23:** 143.
37. Dobrovolskaia-Zavadskaia, N. & M. Rodzevitch. 1941. Panachure artificielle chez des souris irradiées. C. r. Soc. Biol. **135:** 1303.
38. Hance, R. T. 1928. Detection of heterozygotes with X rays. J. Heredity **19:** 481.
39. Murray, J. M. 1927. Studies on the biological effects of roentgen rays on mice. I. Primary general effects. Papers Mich. Acad. Sci. Arts & Letters **8:** 401.
40. Chase, H. B. 1948. Time-factor with respect to X-ray induced greying in the mouse. Genetics. **3:** 100.
41. Chase, H. B., H. Quastler & L. S. Skaggs. 1947. Biological evaluation of 20 million volt Roentgen rays II. Decoloration of hair in mice. Am. J. Roentg. **57:** 359.
42. Chase, H. B. 1949. Greying of hair. I. Effects produced by single doses of X rays on mice. J. Morph. **84:** 57.

EFFECTS PRODUCED ON HAIR BY ATOMIC BOMBS

By Averill A. Liebow
Department of Pathology, Yale University School of Medicine, New Haven, Conn.

Knowledge concerning the effects upon the skin of ionizing radiations from the atomic bombs exploded in Japan is limited for want of accurate data concerning relative dosage of the various components of the gamma ray spectrum and for lack of completely representative material. Specimens of skin (chiefly of scalp) of patients otherwise uninjured, are derived almost exclusively from individuals dying later than the second week after the explosion. Patients surviving for this length of time received less than an erythema dose, since this dose, when delivered to the body as a whole, would have been more rapidly fatal. Regrettably, skin from those dying earlier and who had presumably received a larger dose, is not available.

Clinically, epilation usually appeared rather suddenly, approximately two weeks after the irradiation, chiefly among persons who had been within 1500 yards of the hypocenter (point on the ground above which the bomb exploded). It affected men, women, and children alike. With very few exceptions, the distribution was that of ordinary baldness, the temporal and occipital regions being relatively spared. Even when epilation was almost complete, a few hairs tended to remain in a very sparse arrangement. In a very small number of persons, whom distance or shielding brought to the outer limit of the range of the ionizing radiations, epilation involved only that side presented toward the bomb.[3] The hair of the brows, lids, beard, and body was only rarely lost, despite almost total epilation of the scalp.[4] Almost all patients whose death could be attributed directly to radiation effects, and who lived for more than two weeks, manifested epilation. Some individuals complained of dryness of the epilated skin, a symptom difficult to evaluate, since anhydrosis was not complete. Among survivors, the hair usually began to reappear in some measure between the sixth and eighth weeks. Recovery was progressive, with ultimate restitution of the hair in its former abundance, color, and texture.

The histological observations are based on approximately 30 specimens of scalp of persons dying between the 15th and 42nd days, and on five specimens from the few patients who died of radiation effects, primarily, at a later time. Microscopically, a cessation of differentiation of the internal root sheath was observed. As the trichohyaline-containing layers disappeared, only a narrow column of epithelium continuous with the external sheath was left. Whorled masses of flattened cells, resembling epithelial pearls, were sometimes found in this residual plug. In the region of the matrix, mitoses were now absent, and pigment was irregularly distributed throughout the squamous epithelium in coarse clumps. A small space appeared between the inner end of the epithelial mass, whose bulbous shape had become lost, and the papilla. Likewise, the latter was shrunken and less vascular than usual. If the hair shaft had not as yet been extruded, it remained in attachment near the mouth of the follicle as a "club hair," and there was a parakeratotic transition from the base of the follicle without the intervention of the trichohyaline layer. Vacuoles appeared between the residual epithelium and the glassy sheath, as the former retreated toward the surface. Concomitantly, the glassy sheath became enormously thickened and folded. Located externally to the glassy sheath was the now greatly thickened cellular fibrous connective tissue layer.

Sebaceous glands associated with such follicles were also atrophic, with almost total disappearance of the vacuolated cells. This, however, cannot be attributed directly to irradiation, since similar changes occur in association with atrophic processes, of whatever causation,[1] in the hair follicle. As a rule, the sweat glands retained their typical structure, but, in some instances, there was shrinkage of their acini, resulting from atrophy of the component epithelial cells with pyknosis of nuclei, vacuolation of cytoplasm, and thickening of their basement membranes. In a few patients, the epithelium of the surface likewise was decreased in thickness, but it showed no other remarkable changes.

Particularly in those who survived beyond the sixth week, there was evidence of regeneration, manifested by renewed mitotic activity in the matrix, enlargement and increased vascularity of the papilla, and reappearance of trichohyaline with differentiation of a new internal sheath. Thus, the new hair could be seen making its way upwards through the old external sheath towards the surface of the skin.

In summary, the histological changes were similar to those observed in the normal cycle of loss and replacement of hair, except that the glassy and connective tissue sheaths were possibly thicker and the pigment more irregularly distributed in the temporarily atrophic follicles.[1, 2, 6] The process of atrophy in the irradiated subjects was condensed in time and imposed upon a vast number of follicles all at once, with consequent epilation. The baldness was transient, since the survivors were comprised only of those who had received, upon the body as a whole, less than a permanently epilating dose of ionizing radiations.

Bibliography

1. Auburtin, G. 1896. Das Vorkommen von Kolbenbaaren und die Veränderungen derselben beim Haarwiederersatz. Arch. f. mikr. Anat., **47:** 472–500.
2. Hoepke, H. 1927. Die Haut. Der Haarwechsel. In: von Möllendorff, W. Handbuch der mikroskopischen Anatomie des Menschen. III (1): 79–84. Julius Springer. Berlin.
3. Joint Commission for the Investigation of the Effects of the Atomic Bomb in Japan. Medical Report. (To be published.)
4. Liebow, A. A., S. Warren & E. De Coursey. 1949. Pathology of Atomic Bomb Casualties. Am. J. Pathol., **25:** 853–1027.
5. Pinkus, F. 1927. Die normale Anatomie der Haut. Der Haarwechsel. In: Jadassohn, J. Handbuch der Haut—und Geschlechtskrankheiten. I (1): 212–217. Julius Springer. Berlin.
6. Trotter, M. 1932. The Hair. In: Cowdry, E. V. Special Cytology. **I:** 41–65. Paul B. Hoeber. New York.

THE RELATIONSHIP OF THE ADRENAL, THYROID, AND PITUITARY GLANDS TO THE GROWTH OF HAIR*

By Burton L. Baker†

Department of Anatomy, University of Michigan Medical School, Ann Arbor, Mich.

Since hair appears to be one of the most rapidly growing structures of the body, one might expect that it would serve as a sensitive indicator of the modifying action of hormones on general body growth. In this sense, alterations in the rate of growth of hair might be regarded solely as a reflection of any general metabolic changes induced by the hormones. On the contrary, it is possible that hormones modify the growth of hair in a way that is different from the effects which they elicit elsewhere in the body. In other words, is the response of hair to the action of these humoral agents so unique that the piliary system should be regarded as a special target organ? In the following presentation, we shall seek to analyze the effects of hormones on the piliary system in the light of these two alternatives. The rôle played by the secretions of the adrenal, thyroid, and pituitary glands in the regulation of the growth of hair has been investigated chiefly in two forms, the rat and man. Therefore, information which has been derived from study of the rat will be emphasized and an attempt made to correlate these findings with the more limited information which is available concerning the relationship of these glands to hair in man.

I. The Adrenal Gland

A general knowledge of the physiology of the adrenal cortex is prerequisite to an understanding of the important regulatory control which it exerts over the piliary system. Some of the steroid compounds which have been isolated from the adrenal cortex may be classified roughly into three groups on the basis of their physiological effects: (1) electrolyte-regulating, (2) carbohydrate-regulating, and (3) androgenic (FIGURE 1). It is established that the adrenal glands of both rat and man secrete compounds belonging to groups 1 and 2. These groups of hormones may be designated as C-11 non-oxygenated, and C-11 oxygenated steroids, respectively, since they differ with respect to the absence or presence of an oxygen atom at the C-11 position of the sterol nucleus. Eleven-desoxycorticosterone is the best known compound of group 1 and acts chiefly on inorganic metabolism, stimulating the excretion of potassium and retention of sodium. In contrast, the members of group 2 are more potent in regulating organic metabolism. They modify several different processes in carbohydrate metabolism, among these effects being the acceleration of gluconeogenesis. When administered in high doses, they cause a breakdown of protein, with loss of nitrogen by excretion.

---

* The original work included in this review was supported by grants-in-aid from the Division of Research Grants and Fellowships, United States Public Health Service, and from the Upjohn Company.

† The work involving ACTH is being done in collaboration with Dr. Dwight J. Ingle, of the Upjohn Research Laboratories, and Dr. Choh Hao Li, of the University of California. The investigations involving the local application of adrenal compounds and action of estrogens is a collaborative project with Dr. Wayne L. Whitaker of the University of Michigan.

Rather little is known concerning the production of androgenic compounds by the adrenal glands of the adult rat. On the other hand, in man, the adrenal glands do secrete steroids of this type, the level of which may be followed by determination of the 17-ketosteroids in the urine.

It is essential to point out that this grouping of adrenal compounds is empirical and the boundaries between the functions of these substances are by no means sharply defined. For example, 11-desoxycorticosterone will cause some elevation of liver glycogen, if injected in sufficiently high doses. Likewise, the various members of the C-11 oxygenated group vary among themselves in their effects on both organic and electrolyte metabolism, these effects being modified also by the experimental conditions under which they are studied.

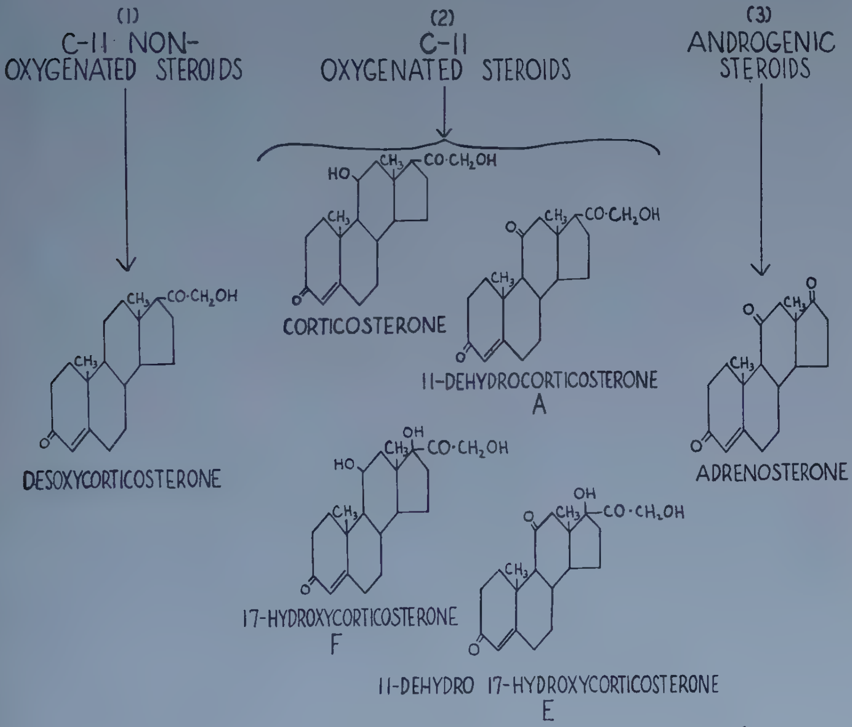


FIGURE 1. Formulae of some of the hormones which have been isolated from the adrenal cortex.

On the basis of several lines of evidence, it appears that, in the rat, the zona glomerulosa and zona fasciculata of the adrenal cortex secrete the electrolyte-regulating and carbohydrate-regulating steroids, respectively. Furthermore, present knowledge indicates that, in the rat, the production of only the latter group is regulated by the anterior hypophysis through its adrenocorticotropic hormone (ACTH). In contrast, most investigators who have studied the problem agree that, in man, the hypophysis regulates the secretion of all three groups of steroids.

1. The Rat. (*a*) *The Effect of Adrenalectomy.* It is of particular interest that when the production of adrenal hormones ceases, due to adrenal ablation, hair grows at a more rapid rate,[10, 31] thus indicating that the adrenal cortex, at its normal level of activity, may inhibit the growth of hair. The epithelium of inactive hair follicles is stimulated to proliferate as early as 2 days following the operation, and, in young underfed rats, hair appears on the surface of the skin within 8 to 9 days.[10] The extent to which the normal wave-like and cyclic pattern of growth is disturbed was studied by Dieke,[15] who observed, in the Norway rat, that the interval between the initiation of new cycles is shortened so that adrenalectomized rats start their 6th cycle at an age when the controls begin their 5th cycle. In our experience, 3 weeks after the operation in normal adult rats, hair is growing much more generally over the body than is normally the case, so that many follicles are activated which otherwise would have remained quiescent.

As would be expected, this accelerated growth is accompanied by important physiologic changes in the skin. Butcher[9] reports a 39 per cent increase in the oxygen consumption of the skin of underfed rats 66 hours after adrenalectomy. Also, Spoor and Ralli,[37] by chemical analysis, confirm the increase in melanin content of the rat skin, following adrenalectomy of rats in which achromotrichia has been induced previously by dietary means. Both the phenol content and reducing power of skin extracts, factors related to melanogenesis, are increased under the conditions of their experiments.

In order to evaluate the significance of acceleration in growth after adrenalectomy, one should know whether the effect is transitory or is maintained. Some evidence is available which indicates that it persists for some time after adrenalectomy. From the data of Dieke,[15] one would infer that a continuous speeding up of the cycles occurs for 20 weeks post-operatively. Our studies of the growth of hair on the dorsum of the neck have brought us to a similar conclusion. In the normal rat, hair usually grows at one time in limited areas which are separated by regions of inactivity. Only occasionally is general growth observed throughout this region. In these studies, observations are made weekly, and the hair is clipped. Since these rats are black-hooded, the presence of growth is demonstrated by pigmentation of the skin even though hair may not be visible grossly.

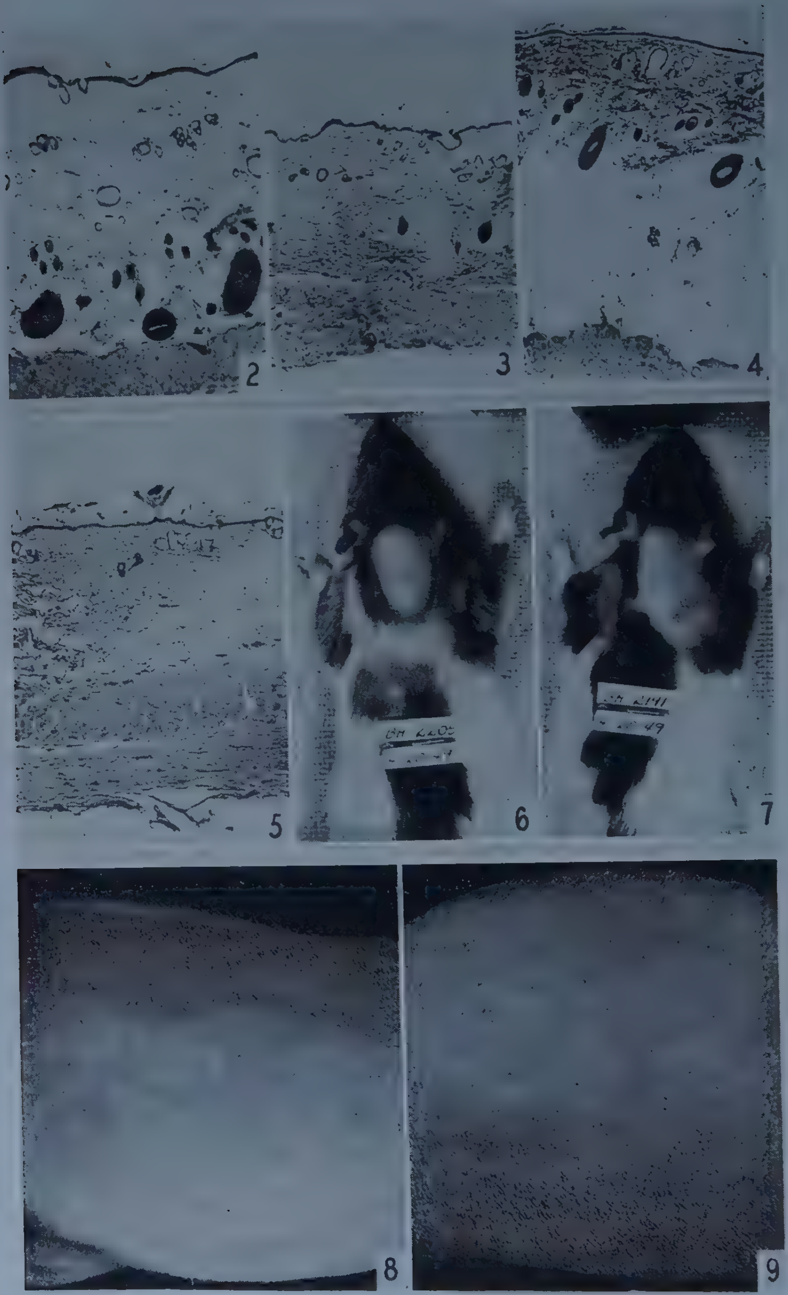
Some rats reassume the normal patterns of growth shortly after adrenalectomy. In many of these animals, however, general and continuous growth throughout the area is indicated by the constant presence of cutaneous pigmentation for a period of 11 weeks after the operation. This pigmentation frequently is accompanied by general growth of hair. We never observe similar evidence of persistent general growth of hair in normal rats. Thus, although there is considerable individual variation, a state of accelerated growth may be maintained for a number of weeks after adrenalectomy. Probably, some tapering off of the effect should be expected if observations are continued for a sufficiently long period of time. The part played by the development of accessory adrenocortical tissue in the early recovery of a normal pattern and rate of hair growth in some of our rats remains to be clarified.

A discordant note is provided by Stein and Wertheimer,[38] who report an increased loss of hair after adrenalectomy of rats. Accelerated growth might be responsible for this observation. They related this effect to loss of the medulla and found various cortical substances incapable of altering this outcome. However, the increased loss of hair was accompanied by ulceration of the skin. Ulceration of the skin is never observed in our adrenalectomized rats and, to our knowledge, has not been reported by others. Hence, one suspects that uncontrolled factors may be involved in the experiments of these investigators.

(*b*) *The Effect of Experimental Hyperadrenocorticism.* Since the C-11 oxygenated steroids are capable of setting up a negative nitrogen balance and retarding general body growth when their concentration in the body is elevated above the normal level, one might expect that they would retard the growth of hair also. Such a condition may be induced by the injection of purified adrenocorticotropin, which increases the production of C-11 oxygenated steroids by the adrenal gland. Treatment of adult male rats with 1 to 3 mg. daily for 21 days results in practically complete suppression of growth of hair, this effect becoming evident during the third week of treatment.[4, 29] The development of most hair follicles is arrested in the epithelial bud stage. In addition, the tela subcutanea is completely depleted of fat, the epidermis is thinned, and dermal connective tissue becomes more compactly arranged (FIGURES 2 and 3). Alkaline phosphatase activity is still present.

Some attempts have been made to elicit similar effects by the parenteral administration of adrenocortical compounds and extracts. No detailed studies are available of the action of pure C-11 oxygenated steroids on the piliary system when given orally or parenterally, although Wells and Kendall[41] point out that, in male rats receiving 11-dehydro-17-hydroxycorticosterone in the drinking water, the fur becomes rough and thin. Since desoxycorticosterone acetate (DCA) has little effect on organic metabolism, one would not expect it to be a very potent inhibitor of growth of hair. In fact, Emmens[18] observes daily doses of 1 mg. of this compound, or implantation as pellets, to be ineffective in the rat. Likewise, Houssay and Higgins[23] report daily injection of doses of DCA, ranging from 0.1 to 1.0 mg., to have only a slightly retarding effect on the regrowth of hair in gonadectomized mice of the CBA and C57 strains. On the contrary, Ralli and Graef[32] find 0.5 mg. given daily to be sufficient to suppress growth of hair under different experimental conditions in rats. All workers are agreed that currently available extracts are not very potent inhibitors. Ralli and Graef find such extracts less effective than DCA, and, in our experience,[25] as much as 5 cc. of Upjohn Lipo-adrenal extract a day fails to suppress growth of hair in force-fed rats. It appears that these unimpressive results may be due to insufficient dosage since one rat which was given the equivalent of 50 cc. of beef adrenal extract a day by stomach tube for 6 weeks exhibits marked suppression of the piliary system (FIGURES 8 and 9). Of particular interest again is the complete mobilization of subcutaneous fat (FIGURES 4 and 5).

(*c*) *Local Action of Adrenocortical Compounds.* When adrenocortical extract



FIGURES 2–9 (*see facing page*).

is applied to the skin, the growth of hair is arrested, but only in the area of application. In our experiments, the dorsum of the neck is studied, with the hormone being applied caudal to the right ear. The pattern of growth is sketched weekly, and the hair is clipped. In rats which are young and healthy, this pattern is symmetrical. Hence, failure of hair to grow on the right side is indicative of inhibition and, according to our criteria, in order to be considered significant, this state must be maintained for 3 consecutive weeks. FIGURE 6 illustrates a control to which alcohol was applied, and FIGURE 7, one receiving 0.1 cc. daily of hog adrenal extract.* Inhibition, as indicated by the absence of cutaneous pigmentation, is observed as early as 8 days after the beginning of treatment. Complete local inhibition in male rats may be maintained for about 160 days. In two rats treated for 180 days, however, hair began to grow during the last two weeks of the experiment, in spite of continuation of treatment (FIGURE 13). These are the only animals which have received extract for so long a period of time, and clearly demonstrate that a refractoriness to the local inhibiting action of extracts will develop.

Here, as is the case after systemic treatment with ACTH, samples of skin, taken from areas treated for comparatively short periods, show the hair follicles to be in the epithelial bud stage.[3] This finding, supplemented by the many weekly observations we have made of the changing patterns of hair growth, indicates that percutaneously applied adrenal hormones are probably more highly inhibitory on follicles in this stage than on those which are growing. The explanation for this observation may rest chiefly on accessibility of the epithelial cells to the surface of the skin, since epithelial buds are in the superficial part of the dermis, in contrast to the growing bulbs which reach into the subcutaneous tissues. Following cessation of treatment, growth of hair is reinitiated promptly, and, subsequent to the second week, a heavy coat grows throughout the area previously treated (FIGURE 18). Subsequently, the normal patterns of growth are regained.

Accompanying this interruption in the growth of hair are certain other changes in the skin, which show that hair is but one of many cutaneous structures affected by the adrenal hormones. If treatment with extract is continued for about 81 days, the epidermis is usually thinner in the area

---

* The extract used throughout our studies dealing with direct application to the skin is specially prepared by Dr. William J. Haines of the Upjohn Company, to whom we express our appreciation. It is dissolved in 25 per cent alcohol and, by the liver glycogen test, is equivalent to 1 mg. of 11-dehydro-17-hydroxycorticosterone per cc.

FIGURE 2 (*see opposite page*). Skin of rat, control for animal illustrated in FIGURE 3.

FIGURE 3. Skin of rat which had received 3 mg. of ACTH daily for 21 days. The hair follicles are inactive, epidermis is thinned, dermis condensed and tela subcutanea depleted of fat.

FIGURE 4. Skin of rat shown in FIGURE 8. Hair is growing. The panniculus adiposus is thick because the oil solvent for the extract was fed by stomach tube.

FIGURE 5. Skin of rat illustrated in FIGURE 9. This animal was given cortin by stomach tube. Hair follicles are inactive. Subcutaneous fat is completely mobilized.

FIGURE 6. Symmetrical pattern of hair growth on a rat treated percutaneously caudal to right ear with 0.1 cc. of 25 per cent alcohol daily for 36 days.

FIGURE 7. Cessation in growth of hair caudal to right ear due to treatment in this area for 36 days with adrenal extract the daily dose of which by the liver glycogen test is equivalent to 100 μg. of 11-dehydro-17-hydroxycorticosterone.

FIGURE 8. One week's growth of hair, control for rat shown in FIGURE 9.

FIGURE 9. Failure of hair to grow on a rat during the sixth week of treatment with adrenal extract. The preparation was fed by stomach tube and consisted of both hog and beef extract, the daily dose being equivalent to 50 cc. of beef adrenal extract.

of treatment in both sexes. In males, the size of the epidermal cells is reduced (FIGURE 10).[11] Likewise, some reduction in thickness of the dermis is observed (FIGURE 11) which is not due to the mere absence of growing hair, since, under normal conditions, there is not a statistically significant difference in thickness of the dermis between an area of active hair growth and one of inactivity (FIGURE 12).

EFFECT OF LOCAL TREATMENT WITH 100 μG. DAILY OF 11-DEHYDRO-17-HYDROXYCORTICOSTERONE OR ITS EQUIVALENT IN HOG ADRENAL EXTRACT ON EPIDERMAL CELL SIZE

Sex	*No. of rats*	*Treatment*	*Mean days of treatment*	*Mean area* (μ^2)		"*t*"
				Left	*Right*	
Male	7	ACE; E*	91±28.4†	42.9±3.0	30.1±5.3	5.58 P <.01
Male	4	Alc.	77±34.3	43.6±6.2	41.3±4.8	0.59 P >.50
Female	8	ACE; E	81±23.4	38.6±4.5	36.4±5.5	0.88 P >.10
Female	6	Alc.	84±11.6	37.8±3.0	38.2±3.0	0.20 P >.50

* ACE = Hog adrenal extract; E = 11-dehydro-17-hydroxycorticosterone.
† Standard deviation.

FIGURE 10.

EFFECT OF TREATMENT WITH EXTRACT, 11-DEHYDRO-17-HYDROXYCORTICOSTERONE, AND 17-HYDROXYCORTICOSTERONE ON DERMAL THICKNESS

Sex	*No. of rats*	*Treatment*	*Mean days of treatment*	*Mean thickness* (μ)		"*t*"
				Left	*Right*	
Male	19	ACE; E; F*	49±37†	554±150.2	467±167.1	1.68 P =.10
Male	4	Alc.	57±31	400± 87.6	390± 87.6	0.09
Female	22	ACE; E	57±26	351± 76.4	280± 69.0	3.20 P <.01
Female	3	Alc.	87±10	288± 38.4	277± 52.2	0.28

* ACE = Hog adrenal extract; E = 11-dehydro-17-hydroxycorticosterone; F = 17-hydroxycorticosterone.
† Standard deviation.

FIGURE 11.

If treatment with this extract is prolonged, atrophy involving all parts of the skin may be induced. These changes are illustrated by samples of skin taken from the right and left sides of the neck of a male rat treated with concentrated extract for 112 days. The epidermis is thinned (FIGURES 16 and 17), and the hair follicles and sebaceous glands are atrophic. The dermis is much thinner and exhibits a loss of discreteness in the outline on the collagenous fibers, these appearing to be fused together. A reduction occurs in the number of fibroblasts, mast cells, and other cellular components of the connective tissue. Glycoprotein in the basement membranes of the epidermis, hair follicles, and sebaceous glands and in the arrectores pilorum muscle remains prominent. Elastic fibers are more concentrated. Subcutaneous fat may be wiped out. Do these structural modifications represent permanent damage? Further study will be required in order to answer this question, but the resumption in hair production, regardless of continued local treatment with extract, which was alluded to previously, indicates that the changes described are not irreversible.

Similar inhibition in the growth of hair may be induced by several pure adrenal compounds. For some time, we have been attempting to compare the growth-inhibiting power of the crystalline compounds, using the technique of local application. One might expect that this characteristic would parallel the action of the steroids on organic metabolism, as illustrated by their capacity to increase the glycogen content of liver. The steroids* being studied, listed in the order of their capacity to increase liver glycogen and promote the work output of muscle, include: 17-hydroxycorticosterone (F), 11-dehydro-17-hydroxycorticosterone (E), corticosterone, 11-dehydrocorticosterone acetate (A), 11-desoxycorticosterone acetate, and 17-hydroxy-11-desoxycorticosterone (S).

THE RELATIONSHIP OF THE PRESENCE OR ABSENCE OF GROWTH OF HAIR AND OF SEX TO THICKNESS OF THE DERMIS

Sex	*No. with no hair growth*	*Mean thickness (microns)*	*No. with hair growth*	*Mean thicknes (microns)*	*"t"*
Males	11	511.1±196*	12	542.1±130.3	0.45 P >.05
Females	14	335.1± 73	11	353.4± 80.2	0.60 P >.05
		"t" = 3.11 P <.01		"t" = 4.13 P <.01	

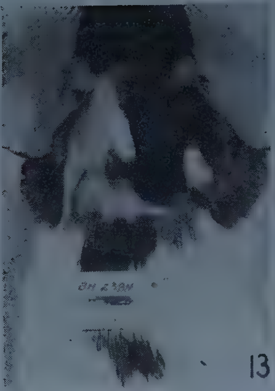
* Standard deviation.

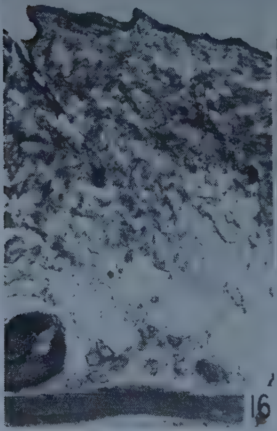
FIGURE 12.

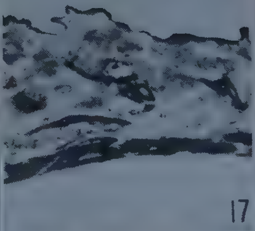
This investigation is still incomplete because of the many unexpected problems which arose. Nevertheless, it is clear from our studies that the C-11 oxygenated steroids are more potent growth inhibitors than the C-11 non-oxygenated group. Furthermore, it appears that 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone are the most potent, with the former hormone being somewhat superior. With these compounds, inhibition may be induced by daily doses in the neighborhood of 10 μg. Thus, in so far as our comparative studies have progressed, there appears to be some parallelism between the capacity of a compound to increase liver glycogen and inhibit growth, and that possibly these two actions may be dependent on a common process. It should be recalled that the C-11 oxygenated compounds are capable of inducing a negative nitrogen balance when administered parenterally. Whether the loss of nitrogen is due to

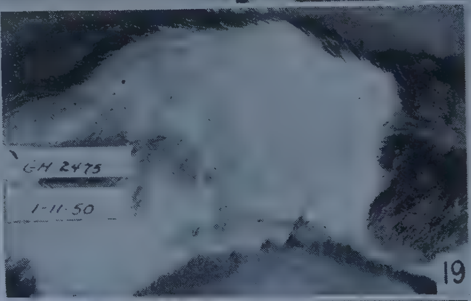
---

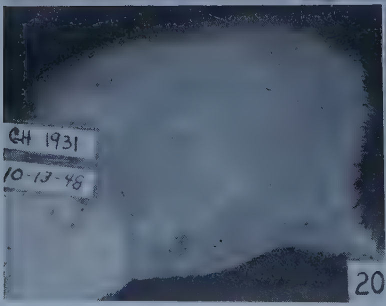
* We wish to express our appreciation to Drs. M. H. Kuizenga and W. J. Haines of the Upjohn Company for 17-hydroxycorticosterone, to Dr. J. M. Carlisle of Merck and Company for 11-dehydrocorticosterone acetate, and to Dr. Edward Henderson of the Schering Corporation for 11-desoxycorticosterone acetate.

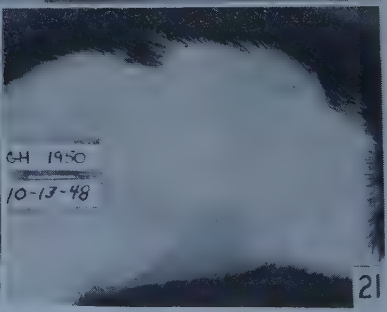












Figures 13–21 (*see facing page*)

excessive catabolism or impaired anabolism is not known definitely, but the recent work of Engel[19] indicates that the adrenal compounds act directly on protein to cause its breakdown. From a chemical standpoint, the presence of an oxygen atom at the C-11 position, and of a hydroxyl group at the C-17 position of the sterol nucleus, seems to have an important relationship to the growth-inhibiting activity of the compound.

The activity of a compound, when administered percutaneously, is conditioned by the vehicle in which it is dissolved. As is true of the sex hormones,[17] absorption seems to be facilitated if volatile organic solvents are employed. For example, whereas desoxycorticosterone acetate and 11-dehydrocorticosterone show some local growth-inhibiting action when administered in alcohol, they are completely inert if the same doses are applied in oil.

(*d*) *Relationship to Other Inhibiting Factors.* Reduction of food intake,[10] maintenance on diets deficient in the filtrate factors of the vitamin B complex,[31] and administration of high doses of estrogen[5, 22, 44] will retard growth of hair in the rat. Involvement of the adrenal cortex is indicated by the fact that, in each of these circumstances, if adrenalectomy is performed, growth of hair is resumed in spite of continued subjection of the rat to the original inhibiting factor[5, 10, 31] (FIGURES 20 and 21). This stimulation is indeed dramatic. Repeatedly in our laboratories we have seen rats debilitated and sick from the toxic effects of intense treatment with estrogens but with hair growing profusely all over the body as a result of adrenalectomy performed 3 weeks previously.

We are seeking to discover the precise manner in which the adrenal gland participates in the suppression of the piliary system in these situations, focusing our attention chiefly on the action of estrogen. It is entirely possible that a similar mechanism comes into play if the animal is denied food or B vitamins.

Many lines of investigation show that systemic administration of estrogens, particularly in high doses, will stimulate the anterior hypophysis to release ACTH and thus activate the adrenal cortex. Hence, when estrogen is observed to be incapable of maintaining suppression of hair growth after

---

FIGURE 13 (*see opposite page*). Growth of hair is resumed in spite of daily treatment with extract on the right side of the neck for 180 days.

FIGURE 14. This rat was treated daily percutaneously caudal to the right ear with 100 μg. of alpha-estradiol until generalized inhibition of hair growth was induced. Adrenalectomy was performed and treatment continued. This growth during the third post-operative week shows no inhibition by estrogen in the area of treatment.

FIGURE 15. This rat was treated similarly to that shown in FIGURE 14 except that adrenal extract was substituted for the estrogen. Local inhibition is induced in spite of adrenalectomy. This is the rat shown in FIGURE 7 but three weeks later, adrenalectomy having been performed on the date of the previous photograph.

FIGURE 16. Skin from left, non-treated side of neck. The specimen shown in FIGURE 17 was taken from the right side of the neck of the same rat.

FIGURE 17. Skin from right side of neck in area treated daily with 0.1 cc. of adrenal extract for 112 days. Hair follicles, epidermis and sebaceous glands are atrophic. The dermis is thinned and subcutaneous fat is completely absent.

FIGURE 18. Re-growth of hair on the right side of neck during the fifth week after cessation of daily treatment with 100 μg. of 11-dehydro-17-hydroxycorticosterone. The rat was treated for 6 weeks, during this period growth of hair being inhibited completely in this area for 4 consecutive weeks.

FIGURE 19. Growth of hair on the side of the body during the twenty-fifth week after hypophysectomy.

FIGURE 20. Failure of hair to grow during seventh week of daily subcutaneous injection of 50 μg. of alpha-estradiol dipropionate.

FIGURE 21. This rat was treated in the same manner as that shown in FIGURE 20, except that adrenalectomy was performed 3 weeks previously. Growth of hair is resumed in spite of continued treatment with the estrogen.

adrenalectomy, one might assume that the anterior hypophysis and adrenal cortex mediate the growth-inhibiting action of estrogens (route D of FIGURE 22). The problem is much more complex, however, and the other possible mechanisms illustrated in this figure must be considered.

If estrogens can inhibit growth of hair by direct action on the hair follicle (A), then the theory of mediation by the adrenal cortex could be abandoned. Williams, Gardner, and DeVita[42] report inhibition to follow percutaneous application of estrone to the skin of the dog. We have attempted to reproduce these results in the rat by using alpha-estradiol and estrone in alcoholic solutions, with and without the addition of small amounts of sesame oil. The doses employed range from 1 to 100 μg. per day. The technique of local application used in the study of adrenal hormones is followed here, and, in addition, the growth of hair is observed on the side of the body for evidence of systemic inhibition. The estrogen is absorbed into the blood stream and the higher doses produce a generalized retardation in growth throughout the areas observed. Although hair frequently does not grow in the area of application, failure of hair to grow elsewhere makes it impossible for us to detect a clear-cut local action

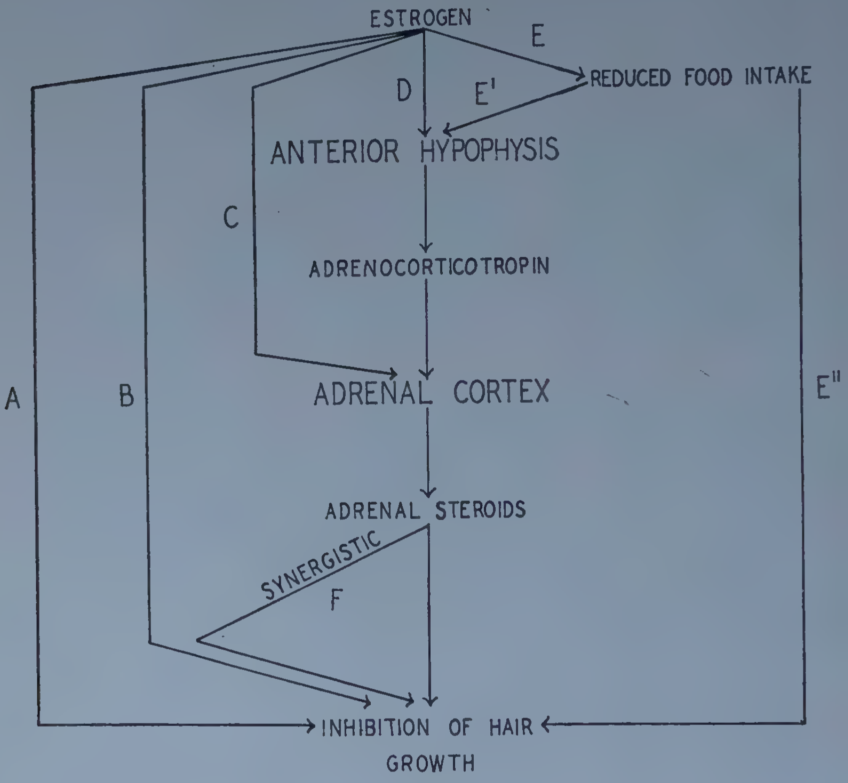


FIGURE 22. Possible ways in which estrogen might inhibit the growth of hair.

by this procedure. Prolongation of treatment does not always maintain inhibition in the area of treatment. Thus, it is shown that, under these conditions, a local inhibitory action by estrogen cannot be divorced from its systemic effects. The possibility exists that the generalized suppression of growth of hair may mask the local effect of the estrogen.

Does estrogen inhibit growth of hair indirectly by causing a reduction in food intake (route E of FIGURE 22)? Hooker and Pfeiffer,[22] in paired feeding experiments, conclude that reduction in food intake is not prerequisite to the effectiveness of estrogen. When estrogen is administered by injection in our experiments, it suppresses growth of hair in rats fed by stomach tube, so that this factor may be eliminated.

Finally, do adrenal hormones facilitate or potentiate the growth-inhibiting action of estrogens in the peripheral tissues (routes B and F of FIGURE 22)? The critical work of Ingle first demonstrated the potentiating action of adrenal secretions on the diabetogenic action of estrogen. Subsequent investigations by him and others since have led to the development of one of the most important basic concepts in the field of adrenal physiology—that adrenal hormones serve to prepare the body tissues for mobilization by the action of estrogen or other agents. In collaboration with Dr. Dwight J. Ingle of the Upjohn Research Laboratories, we are examining this hypothesis with respect to the action of estrogen on hair, by attempting to suppress growth of hair in force-fed adrenalectomized rats by injecting estrogen along with adrenal extract, the latter being injected at a dosage which, by itself, is not significantly inhibitory. These studies are not completed, but as of the moment, estrogen is proving to be effective in inhibiting growth of hair in the adrenalectomized rat, if an optimal amount of extract is administered concurrently.

Thus, it appears that the adrenal cortex may act to make the peripheral tissues responsive to the growth-retarding action of estrogen. This conclusion does not eliminate the possibility that some of the inhibition of growth which follows parenteral administration of estrogen is mediated by the hypophysis and adrenal cortex. Another disturbing factor remains. This is the failure of large doses of estrogen to maintain inhibition locally in the rat by percutaneous application. Can it be that, in these experiments, the endogenous production of adrenal hormones is insufficient to keep their concentration high enough in the area of application to facilitate the action of estrogen? The essential rôle played by adrenocortical hormones in the possible local growth-inhibiting action of estrogen is illustrated by the following experiment. Adrenal extract in amounts equivalent to 100 μg. of compound E was applied daily to the skin caudal to the right ear in one group of rats. A second group received 100 μg. of alpha-estradiol daily in a similar manner. Treatment was continued until inhibition of growth of hair was observed in both groups. As pointed out previously, the growth retardation was general in the estrogen-treated animals and local in those given extract. Then adrenalectomy was performed and treatment continued. In spite of the stimulation of growth which followed, the extract maintained a state of inhibition in the treated area (FIGURE 15), whereas the estrogen was completely ineffective (FIGURE 14).

(*e*) *Specificity of Inhibition by Adrenal Hormones.* The inhibition in growth of hair which occurs during systemic treatment with ACTH and adrenal compounds appears to be an over-dosage effect. Likewise, when growth is inhibited locally by direct application of hormones to the skin, it is probable that an abnormally high concentration of hormones is created in that area. If inhibition results from the production of such an abnormal physiological state, then one must consider the possibility that the effect is non-specific. Certainly, no definite solution to this problem, with respect to the adrenal hormones, is now apparent and space does not permit consideration of all pertinent investigations. The following evidence might be cited, however, which shows that the regulatory influence of adrenal hormones over growth of hair is at least different from that exerted by other hormones. First, in so far as is known, the removal of no other endocrine gland results in over-growth of hair in the rat. Also, this finding may be interpreted to indicate that the normal level of adrenal hormones exerts a retarding influence on the growth of hair. Second, since adrenal hormones are locally active in inhibiting growth of hair without producing systemic effects when applied to the skin, the chance that other glands or organs may be the mediators of a non-specific action is eliminated. Admittedly, the possibility still exists that they might participate indirectly in a non-specific effect. Third, the varying potencies of the adrenal compounds, in inhibiting growth locally, suggest the existence of a correlation with the chemical structure of the hormones. Fourth, to our knowledge, there is no other substance or group of substances which will stop the growth of hair locally without damaging the skin. Testosterone propionate, cholesterol, cholic acid, and thyroxine, in concentrations considerably above the minimal effective dose of the C-11 oxygenated steroids, are not inhibitory. Estrogens stand as a possible exception, but the hypothesis that they, too, inhibit growth by local action requires additional investigation.

(*2*) *Man.* Information derived from the study of the relationship of the adrenal glands to the piliary system in lower mammals cannot be transferred to man without considerable modification. This situation is due to the greater control exerted by sex hormones over the growth and distribution of hair in man and probably, also, to the capacity of the human adrenal cortex to produce substances identical or physiologically similar to the sex steroids. No attempt will be made here to survey the extensive array of clinical syndromes accompanied by disturbance of the piliary system in which there is possible involvement of the adrenal gland. Rather, we shall attempt only to draw some general conclusions from this body of knowledge.

Growth of pubic, axillary, and facial hair may be regarded as a secondary sex characteristic. A state of adrenal insufficiency, whether due to bilateral adrenalectomy[24] or Addison's disease,[26, 30] is accompanied by reduction in the amount of pubic and axillary hair. Two concepts have been presented to explain this occurrence. Albright[1] holds that the production of pubic hair in females is controlled by the "N" hormone (one having androgenic properties) of the adrenal cortex. Some of the evidence marshalled by him follows. (1) Patients with primary ovarian insufficiency have reduced axillary and pubic hair and a low 17-ketosteroid excretion. (2) Administration of estrogen therapy to such patients restores this hair, supposedly by stimulating the pituitary-adrenal axis and the output of "N" hormones by the latter gland. (3) The topical application of testosterone to the axilla will stimulate the growth of hair in the area of treatment. (4) The 17-ketosteroid excretion rises at puberty in females and may be considered to be an index of the secretion of androgenic steroids by the adrenal cortex. Thus, the growth of hair at this time may be controlled by the adrenal gland rather than by the ovaries. (5) Besides Addison's disease, in panhypopituitarism, myxedema, and old age, loss of pubic and axillary hair occurs in association with a low excretion of 17-ketosteroids in the urine.

A second concept, presented by Mussio Fournier *et al.*,[30] suggests that estrogens control growth of hair in the pubic and axillary areas, but that their action must be synergized by adrenocortical hormones. This conclusion is based on the greatly reduced pubic and axillary hair observed in a woman with Addison's disease, but who presented evidence of continued production of estrogens by the ovary. Also, a case of Addison's disease with similar characteristics is described by Kepler *et al.*[26] The growth of pubic and axillary hair in eunuchs castrated before the time of puberty[26] is pertinent.

In contrast to this loss of hair resulting from adrenal insufficiency is the hypertrichosis which accompanies clinical syndromes involving hyperactivity of the adrenal cortex. Adrenocortical neoplasms may produce this condition. Cushing's disease is accompanied by masculine distribution and overgrowth of hair in women and boys. In the adrenogenital syndrome of women, one may find growth of hair on the upper lip, chin, and other areas of the body, baldness, and assumption of the male configuration by the pubic hair.[34] Excessive androgen production by the adrenal glands is regarded as the cause of this situation. A satisfactory correlation of these findings with those described previously in animals is most difficult at this time. The picture is complicated by the development of mild hirsutism in cases of rheumatoid arthritis treated with 11-dehydro-17-hydroxycorticosterone.[21] This compound is not known to be strongly androgenic in rodents, and the stimulation of growth of hair in man is quite contrary to its inhibitory action in lower mammals. Therefore, it may be expected that, when injected into man, (1) 11-dehydro-17-hydroxycorticosterone is converted into another substance having androgenic properties, (2) that the endogenous steroid hormone secretion is unbalanced in favor of greater secretion of substances with androgenic properties, or (3) that the hair follicles of man respond to this compound in an entirely different way than do those of the rat.

An additional point of difference between man and rodents, with respect to the relationship of the adrenocortical hormones to hair, is the failure of these substances to inhibit the growth of hair when applied percutaneously to human beings. In our hands, adrenal extract in alcoholic solutions or in a cream is ineffective. One must keep in mind, however, that there is no assurance that, when applied percutaneously, comparable amounts of hormone reach the growing parts of the hair follicle in man, as compared with the rat. In man, the skin is much thicker and the cyclic nature of hair growth is different, so that the difficulties attendant upon effective therapeutic use of adrenal compounds, in controlling hypertrichosis, are amplified greatly.

II. The Thyroid Gland

Thyroidectomy of animals causes a reduction in the rapidity with which hair grows back after clipping.[12, 13, 18] Furthermore, Dieke[15] has observed a progressively greater delay in the time of initiation of successive cycles, accompanied by a reversal in direction of spread of the wave of growth. Administration of thyroxine to thyroidectomized rats restores the normal rate of growth but, if this hormone is given in excess, then suppression results from the hyperthyroid state so induced.[12] If hair follicles are brought to a state of inactivity by underfeeding, thyroxine will reactivate them.[7] Chang reports that administration of thyroxine to intact rats exerts a variable effect on growth of hair, but if treatment is continued long enough, suppression will occur.

Since there is uniform agreement that thyroidectomy retards growth of hair, it might be expected that administration of antithyroid drugs, which induce a state of physiological thyroidectomy, would have a similar effect. The studies of Dieke,[14] however, demonstrate a great divergence in the effects of these drugs on the piliary system. Thus, in the doses employed, thiourea has no effect, phenyl thiourea suppresses pigment formation without affecting growth of hair, while alpha-naphthyl thiourea suppresses both hair and pigment formation.

Does the thyroid hormone act directly on the hair apparatus, or by primary modification of the nutritional state of the animal? Observations in our laboratory indicate that different strains of rats vary widely with respect to the magnitude of the retardation in growth of hair after thyroidectomy. This difference seems to be correlated with the natural vigor of the animal. For example, the post-thyroidectomy retardation in growth of hair in the Long-Evans strain is minimal, whereas many Wistar rats grow very little hair after this operation. In our laboratory, the former is much the hardier of the two strains. Chang,[12] on the basis of very limited observations, concludes that the thyroid hormone acts directly on the hair follicle since treatment of under-nourished rats accelerates the rate of growth of hair in spite of continued loss in body weight. Additional study is needed to clarify this point.

III. The Hypophysis

Hypophysectomy of the rat produces a considerable alteration in the normal development of the pelage. Smith[35] first pointed out that the hair of these animals remains infantile in type. A general slowing down in the rate of growth is demonstrated by Dieke,[15] who observes a progressively greater delay in the time at which successive cycles begin. Snow and Whitehead[36] find the weight of hair produced per sq. cm. of body surface to be 70 per cent of normal. These observations are impressive, but it should be emphasized that the hypophysectomized rat does grow a considerable amount of hair, as shown by FIGURE 19, which illustrates the growth of hair which occurred during the twenty-fifth week after hypophysectomy. In general, the wave-like pattern of growth which characterizes the normal rat is retained for some time. Replacement therapy with crude extracts containing the growth hormone or implants of the hypophysis will restore the rate and character of growth of hair to normal[35, 36, 40] and, according to Snow and Whitehead, the amount of hair produced may be increased beyond normal. Pituitary extracts have not proven to be of significant value in the treatment of some types of alopecia in man.[27]

Discussion

As pointed out in the introduction, it is desirable to ascertain whether the modifications induced in growth of hair by these hormones are specific effects, or only reflections of general metabolic alterations. In general, variations in the rate of hair and body growth run parallel in most of the experimental situations which have been described. Thus, in rats, growth of both hair and other parts of the body is retarded in states of hypothyroidism, hyperthyroidism, hypopituitarism, and hyperadrenocorticism. Hence, it may be concluded that most of these responses to endocrine influences are reflections of general metabolic actions. On the contrary, two reactions stand out which seem to place the responsiveness of hair in a unique category. The first is the acceleration in rate of growth of hair which follows adrenalectomy. This occurs regardless of the physical condition of the animal. One has only to observe the prolific growth of hair on a sick adrenalectomized rat to be impressed with this fact. In contrast, adrenalectomy in young rats stops general body growth.[20] In only two other tissues, lymphoid tissue[33] and the epiphyseal cartilage of the tibia,[43] has evidence of overgrowth after adrenalectomy been observed.

Concerning this response on the part of lymphoid tissue, there is disagreement,[39] and, in the case of cartilage, it is not as impressive as in the case of hair. Thus, in the rat, hair responds to adrenalectomy in a way that has no exact parallel among the other body tissues. Prerequisite to an accurate analysis of this problem is the availability of more precise information regarding the rate of mitotic proliferation in the growing part of the hair follicle, as compared with that in other tissues. Thus, is there a tendency towards overgrowth in all tissues of the body after adrenalectomy, with the effect becoming most evident in those tissues which normally exhibit the highest rate of cell division?

Secondly, that hair should grow so extensively, even though at a reduced rate in the hypophysectomized rat, seems unusual in view of the very low level of proliferation which characterizes other parts of the body. Can it be that hair continues to grow because hypophysectomy causes atrophy of the zona fasciculata in the adrenal cortex, reducing production of the C-11 oxygenated steroids, and thus creating a state of partial adrenal insufficiency?

In conclusion, one may expect that any agent which retards or promotes growth of the body as a whole will exert parallel effects on hair. Nevertheless, there seems to be sufficient individuality in the response of hair to some hormonal imbalances for future research to be directed profitably toward discovering the extent to which the hair apparatus should be regarded as a special end-organ for at least some of the hormones which affect it.

Bibliography

1. Albright, F. 1947. The effect of hormones on osteogenesis in man. Recent Progress in Hormone Research **1:** 293. Academic Press. New York.
2. Baker, B. L. 1949. Anatomical modifications induced by adrenocortical secretions with special reference to the regulation of growth. A.A.A.S. Symposium on the Adrenal Cortex. In press.
3. Baker, B. L. & W. L. Whitaker. 1948. Growth inhibition in the skin following direct application of adrenal cortical preparations. Anat. Rec. **102:** 333.
4. Baker, B. L., D. J. Ingle, C. H. Li, & H. M. Evans. 1948. Growth inhibition in the skin induced by parenteral administration of adrenocorticotropin. Anat. Rec. **102:** 313.
5. Baker, B. L. & W. L. Whitaker. 1949. Relationship of the adrenal cortex to inhibition of growth of hair by estrogen. Am. J. Physiol. **159:** 118.
6. Butcher, E. O. 1937. Hair growth in adrenalectomized, and adrenalectomized thyroxin-treated rats. Am. J. Physiol. **120:** 427.
7. Butcher, E. O. 1940. The effects of irritants and thyroxin on hair growth in albino rats. Am. J. Physiol. **129:** 553.
8. Butcher, E. O. 1941. Accelerated hair growth in the rat after adrenalectomy cannot be attributed to the thyroid. Proc. Soc. Exp. Biol. and Med. **48:** 120.
9. Butcher, E. O. 1943. Oxygen consumption of skin and hair growth after adrenalectomy in the white rat. Endocr. **32:** 493.
10. Butcher, E. O. & R. A. Richards. 1939. The relation of the adrenals to the retarded hair growth in underfed albino rats. Endocr. **25:** 787.
11. Castor, C. W. & B. L. Baker. Unpublished.
12. Chang, H. C. 1926. Specific influence of the thyroid gland on hair growth. Am. J. Physiol. **77:** 562.
13. Chang, H. C. & T. P. Feng. 1929. Further studies on thyroid and hair growth Chin. J. Physiol. **3:** 57.
14. Dieke, S. 1947. Pigmentation and hair growth in black rats as modified by the chronic administration of thiourea, phenyl thiourea and alpha-naphthyl-thiourea. Endocr. **40:** 123.
15. Dieke, S. 1948. The effect of removing various endocrine glands on the hair cycles of black rats. Endocr. **42:** 315.
16. Dorfman, R. I. 1949. The bioassay of adrenal cortical steroids. Ann. N. Y. Acad. Sci. **50:** 556.
17. Emmens, C. W. 1940. The inunction of sex hormones on the skin. J. Endocr. **2:** 368.
18. Emmens, C. W. 1942. The endocrine system and hair growth in the rat. J. Endocr. **3:** 64.
19. Engel, F. L. 1949. Studies on the nature of the protein catabolic response to adrenal cortical extract. Accentuation by insulin hypoglycemia. Endocr. **45:** 170.
20. Hartman, F. A. & G. W. Thorn. 1930. A biological method for the assay of cortin. Proc. Soc. Exp. Biol. & Med. **28:** 94.
21. Hench, P. S., E. C. Kendall, C. H. Slocum, & H. F. Polley. 1949. The effect of a hormone on the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: Compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis; preliminary report. Proc. Staff Meet., Mayo Clinic. **24:** 181.
22. Hooker, C. W. & C. A. Pfeiffer. 1943. Effects of sex hormones upon body growth, skin, hair and sebaceous glands in the rat. Endocr. **32:** 69.
23. Houssay, A. B. & G. M. Higgins. 1949. Hormonal influences upon the rate of growth of hair in gonadectomized mice. Proc. Staff Meet., Mayo Clinic, **24:** 597.
24. Huggins, C. A. & W. W. Scott. 1945. Bilateral adrenalectomy in prostatic cancer. Ann. Surg. **122:** 1031.
25. Ingle, D. J. & B. L. Baker. Unpublished.

26. Kepler, E. J., G. A. Peters, & H. L. Mason. 1943. Addison's disease associated with pubic and axillary alopecia and normal menses. J. Clin. Endocr. **3:** 497.
27. Lord, L. W. 1933. Anterior lobe pituitary extract in the treatment of alopecia. Arch. Derm. & Syph. **28:** 381.
28. Martin, L. & M. Hynes. 1948. Clinical Endocrinology. The Blakiston Co. Philadelphia.
29. Moon, H. D. 1937. Inhibition of somatic growth in castrate rats with pituitary extracts. Proc. Soc. Exp. Biol. & Med. **37:** 34.
30. Mussio Fournier, J. C., E. Pollack, & J. J. Lussich Siri. 1949. Loss of axillary and pubic hair in a patient with Addison's disease and regular menstruation. J. Clin. Endocr. **9:** 555.
31. Ralli, E. P. & I. Graef. 1943. Stimulating effect of adrenalectomy on hair growth and melanin deposition in black rats fed diets adequate and deficient in the filtrate factors of vitamin B. Endocr. **32:** 1.
32. Ralli, E. P. & I. Graef. 1945. The effects of the synthetic and natural hormone of the adrenal cortex on melanin deposition in adrenalectomized black rats fed diets adequate and deficient in the filtrate factors of vitamin B. Endocr. **37:** 252.
33. Reinhardt, W. O. & R. O. Holmes. 1940. Thymus and lymph nodes following adrenalectomy and maintenance with NaCl in the rat. Proc. Soc. Exp. Biol. & Med. **45:** 267.
34. Selye, H. 1947. Textbook of Endocrinology. Acta Endocrinologica. Montreal.
35. Smith, P. E. 1930. Hypophysectomy and replacement therapy in the rat. Am. J. Anat. **45:** 205.
36. Snow, J. S. & R. W. Whitehead. 1935. Relationship of the hypophysis to hair growth in the albino rat. Endocr. **19:** 88.
37. Spoor, H. J. & E. P. Ralli. 1944. Chemical studies on melanogenesis in normal and adrenalectomized rats. Endocr. **35:** 325.
38. Stein, L. & E. Wertheimer. 1941. Hair loss as deficiency test of medullectomy in rats. J. Endocr. **2:** 418.
39. Stoerk, H. C. 1944. Thymus weight in relation to body weight in castrated and adrenalectomized rats. Endocr. **34:** 329.
40. Thompson, K. W. & D. W. Gaiser. 1932. The effect of diet and pituitary growth hormone on hypophysectomized rats. Yale J. Biol. and Med. **4:** 677.
41. Wells, B. B. & E. C. Kendall. 1940. The influence of corticosterone and C17 hydroxydehydrocorticosterone (Compound E) on somatic growth. Proc. Staff Meet., Mayo Clinic, **15:** 324.
42. Williams, W. L., W. U. Gardner, & J. DeVita. 1946. Local inhibition of hair growth in dogs by percutaneous application of estrone. Endocr. **38:** 368.
43. Wyman. L. C. & C. Tum-Suden. 1945. The effect of adrenalectomy on the epiphyseal cartilage of the rat. Endocr. **36:** 340.
44. Zondek, B. 1936. The inhibitory effect of follicular hormone on the anterior lobe of the pituitary gland. Lancet **1:** 10.

PATTERNED LOSS OF HAIR IN MAN: TYPES AND INCIDENCE*

By James B. Hamilton

Department of Anatomy, State University Medical Center at New York City, Brooklyn, New York

This account summarizes certain aspects of a study which is to be published in detail elsewhere and has the following aims:

(1) To establish types or categories of scalp hairiness which can be used as standards for classification and grading of the extent of common baldness.†

(2) To survey the incidence of these scalp types in members of each sex throughout life, beginning with the first appearance of hair in the fetus.

(3) To gain information regarding the relationship of scalp types to factors which influence the development of baldness, in particular to genetic, age, and endocrine factors.

(4) To ascertain if bald men, as compared with men who did not become bald, have higher titers of urinary ketosteroids or a greater development of a secondary sex character, axillary hair, which can be measured quantitatively and used as an index of certain aspects of the endocrine status. This part of the study stems from the fact that common baldness is induced by androgenic stimulation (Hamilton, 1942).

(5) To test if the tendency to acquire baldness of the scalp is associated with a disposition to baldness on another part of the body where alopecia is common, *i.e.*, on the lateral surfaces of the legs.

Materials and Methods

The subjects consisted of 312 males and 214 females who were students or members of the staff of Yale, Missouri, and New York State Universities or were inmates or members of the staff of Riker's Island Prison, of Fairfield State Hospital in Newtown, Connecticut, and of the Jewish Sanatarium in Brooklyn; 83 babies and children attending the Well-Baby Clinic at the Long Island College Hospital and the State Training School of Winfield, Kansas; 77 normal Chinese males in New York; and 62 fetuses and stillborn children at the Carnegie Institute of Embryology in Baltimore, Maryland. All subjects were white, with the exception of the series of Chinese males. The number of subjects in each decade between 20 and 89 years of age is listed separately by sex in FIGURE 25. Fetuses and still-births are described in detail in TABLE 1.

Bioassay of androgens and colorimetric assay of ketosteroids were carried out in urine collected for 96 to 120 consecutive hours from 51 men 21 to 75 years of age. Extraction of urinary steroids and their colorimetric assay were conducted in the manner employed by Dobriner and associates (1948). These findings have been presented in detail elsewhere (Hamilton and Hamil-

---

* This study was supported in part by research grants RG 1036 and H 397 by the U. S. Public Health Service. It is also a pleasure to express appreciation of the technical aid of Gordon Mestler, and of the cooperation and helpfulness of the subjects and staffs of the institutions in which this work was carried out.

† Common baldness is employed as a general term for the common forms of alopecia that, in some dermatological classifications, are subdivided into "idiopathic premature," and "senile" alopecia. Baldness with seborrheic dermatitis is also included. As shown in the data below, these forms of baldness account for more than 99 per cent of the cases of alopecia.

TABLE 1

PILIARY STATUS OF THE SCALP DURING FETAL LIFE

Fetus	*Twins:* (S)*imilar* (D)*issimilar*	*Length (mm.) from crown to rump*	*Sex*	*Body wt. (gms.)*	*Age in weeks for cases in which data are available*	*Hair on surface of scalp*
1	(D)	143	F	240		None visible grossly. Status in twins similar.
2	(D)	141	M	245		
3		144	F	235		Present over entire scalp but much more sparse on occiput than elsewhere.
4		157.7	F	357		Present over entire scalp and more plentiful than in fetus with 144 mm. C.R. length.
5		158	F	275		Present over entire scalp and forehead.
6		160	F	369		Present over entire scalp and forehead, but more sparse on the occiput than elsewhere.
7	(D)	163	M	260		None visible grossly. Status in twins similar.
8	(D)	143	F	200		
9	(D)	163.5	F	373.5		Present over entire scalp and forehead. Status in twins similar.
10	(D)	167	M	408.5		
11	(D)	164	M	348	19 5/7	Present over entire scalp and forehead. Status in twins similar.
12	(D)	163	M	295		
13	(D)	164	F	345	21	Present over entire scalp and forehead. Status in twins similar.
14	(D)	172	M	420		
15		171	M	393		Present over entire scalp and forehead.
16	(S)	172.5	F	428	21	Present over entire scalp and forehead. Status in twins similar.
17	(S)	175.2	F	447		
18		175	F	390		Present over entire scalp and forehead.
19		177.5	M	387	21 2/7	Present over entire scalp and forehead, but hairs are shorter than in the other fetuses of 171–177 mm. C.R. length.
20		182	M	461		Present over the entire scalp, but, with the exception of fetus 26, the hair is shortest of the seven fetuses, 20 to 26 inclusive.
21	(D)	187	F	434	21 6/7	Present over entire scalp and forehead. Status in twins similar.
22	(D)	177.5	M	387		
23	(D)	184	F	440	21 1/2	Present over entire scalp and forehead. Status in twins is dissimilar. The growth of hair is considerably greater in the smaller than in the larger twin.
24	(D)	175	F	390		
25		188	F	461		Present over the entire scalp and forehead, but there is an area of sparseness between the tonsure and the more anteriorly-located scalp.

TABLE 1—(*Continued*)

Fetus	*Twins: (S)imilar (D)issimilar*	*Length (mm.) from crown to rump*	*Sex*	*Body wt. (gms.)*	*Age in weeks for cases in which data are available*	*Hair on surface of scalp*
26		189	F	534		Present over entire scalp and forehead, but short.
27		197	M	581		Present over entire scalp and forehead.
28	(D)	202	F	630	24	Present over entire scalp and forehead. Status in twins similar.
29	(D)	199	F	595		
30		205.3	F	664.5	24 3/7	Present over entire scalp and forehead.
31	(D)	207	F	545		Present over entire scalp and forehead. Extensive and well-developed hair on the body. Status in twins similar.
32	(D)	202	F	510		
33		210	F	645		Present over entire scalp and forehead.
34		212	M	609	24	Present over entire scalp and forehead. Piliary coat is more extensive than in any of younger fetuses and extends over the cheek, back, legs, and chest, but not over the pubis or axilla.
35		217	F	744	25	Present over entire scalp and forehead. Hairs in this fetus and in older specimens have attained considerably greater length and thickness.
36		226.5	M	785	25 5/7	Present over entire scalp and forehead. In this fetus, the hairs on the portion of the forehead superior to lateral borders of eyebrows are almost as long and thick as those on the scalp. On the remainder of the forehead, the increase in size and thickness of the hairs has not kept pace with that of the hair on the scalp. This disparity in growth of hair over most of the scalp, as contrasted with that on the forehead, increased in the still older fetuses.
37	(D)	228	M	860		Present over entire scalp and forehead. Status in twins similar.
38	(D)	230	M	820		
39	(D)	232	F	920		Present over entire scalp and forehead. Status in twins similar.
40	(D)	255	F	1010		
41	(D)	214	M	720		Present over entire scalp and forehead. Very extensive over all of the forehead, although the hairs are not as thick as on the scalp. The piliary coat is also extensive over the cheek, upper lip, back, and extremities, including the digits, but not over the axilla or pubis. Status in the twins similar.
42	(D)	243	F	770		

TABLE 1—(*Concluded*)

Fetus	*Twins:* (S)*imilar* (D)*issimilar*	*Length (mm.) from crown to rump*	*Sex*	*Body wt. (gms.)*	*Age in weeks for cases in which data are available*	*Hair on surface of scalp*
43		232	M	975	26 1/2	Sparse over deep frontal area but plentiful elsewhere.
44	(D) or (S)	240	M	1050	25 1/2	Present over entire scalp and sides of forehead. Status in twins similar.
45	(D) or (S)	236	M	1030		
46	(D) or (S)	250	M			Thin and short over frontal and especially over frontoparietal areas. Status in twins similar, except that the depth of the frontoparietal regions of thin hairs is not identical in the twins.
47	(D) or (S)	266	M			
48	(D)	278	M	1410		Deep frontoparietal (5 cm.) and shallow (2 cm.) frontal areas in anterior border of scalp, on which the hair is much thinner and shorter than on more posteriorly-lying portions of scalp. Status of twins similar.
49	(D)	268	M	1260		
50	(D)	275	F	1450		Uniform over entire scalp in fetus 50. Scalp of fetus 51 as in fetuses 48 and 49. Hence, status in twins dissimilar.
51	(D)	282	M	1535		
52		278	F		31	Malformed head and scalp.
53		301	F		33 1/2	Fine over deep frontotemporal but not over frontal regions.
54		317	M		35	Malformed head and scalp.
55	(D)	327	F	2290	35	Thin, short hairs over deep frontal area. Status of twins similar.
56	(D)	303	F	2090		
57	(D)	334	F	2470	35 1/2	Thin, short hairs over frontal and frontoparietal regions. Status of twins similar.
58	(D)	340	F			
59	(D)	333	M	2015	32	Thin, short hairs over frontal and frontoparietal region. Status of twins similar.
60	(D)	298	M	1530		
61		341	F	2565		Deep frontoparietal areas with thin, short hairs.
62		380	M	3415	40	Thin, short hairs over frontoparietal areas.

ton, 1948). Androgens were assayed biologically, utilizing the chick's comb methods employed by Hollander and associates (1941), and statistical procedures for assays as described by Bliss (1944).

Loss of hair on the lateral surfaces of the legs was classified in five progressive stages, the three most advanced stages of which were considered to represent alopecia, and are the stages of alopecia referred to below. In these three stages, the lateral surfaces of the legs are either entirely bare or have only a fringe of hair in an area not more than 5 cm. in height that lies immediately superior to the ankle.

Results

The following types of scalp categorize the most typical sequences in the development of common baldness by Caucasians and are illustrated by sketches in FIGURE 1. There are, of course, variant patterns and slight modifications of these types of scalp, so that no single sequence is followed uniformly by all subjects.

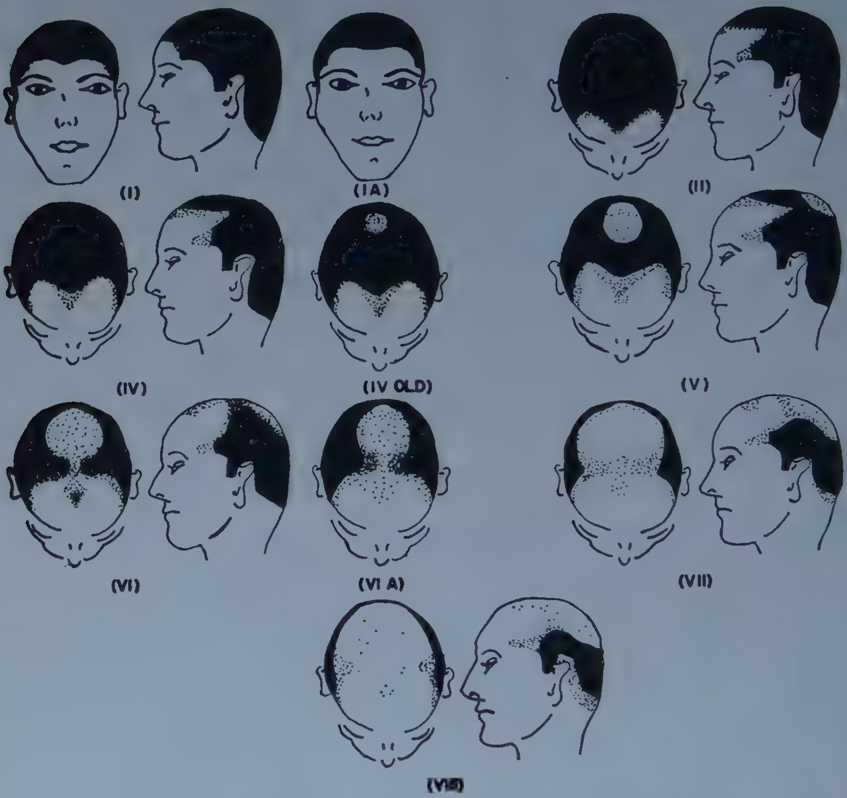


FIGURE 1. Sketches of the categories of scalp hairiness which were set up for classification and grading of the extent of common baldness. The categories or types of scalp are described in the text and illustrated in FIGURES 2 to 15 by photographs of subjects. In scalp Types I, II, and III, the loss of hair is insufficient to qualify as baldness. Types IV, V, VI, VII, and VIII represent a graded progression of common baldness. No sketch of Type III is included in FIGURE 1 because of the variety of conditions that constitute Type III.

Scalps Which Are Not Bald, Types I to III. Type I. The essential feature of Type I is the absence of bilateral recessions along the anterior border of the hair line in the frontoparietal regions.

In this, and in the other scalp types, there is a variant form in which the entire anterior border of the hair line lies high on the forehead. These variants are designated by the letter A. Therefore, this variant of Type I is referred to as Type IA. It is illustrated in FIGURE 1, where it can be seen to have the essential feature of Type I, which is the lack of frontoparietal recession of the hair line.

Type II. The anterior border of the hair line in the frontoparietal regions has triangular areas of recession which tend to be symmetrical. These areas of denudation extend no farther posteriorly than a point 3 cm. anterior to a line drawn in a coronal plane between the external auditory meatuses. Hair is also lost, or sparse, along the midfrontal border of the scalp, but the depth of the affected area is much less than in the frontoparietal regions. A region of midfrontal sparseness, or denudation of such depth that it extends as a band of uniform width along the entire anterior border of the scalp, is so rare that no Type IIA was employed in routine classification.

Type III. To avoid inclusion of disputable instances of baldness, borderline cases are listed separately as Type III. Also included in Type III are scalps in which classification is rendered inaccurate due to scars, lateral asymmetry in denudation, unusual types of sparseness and thinning of the hair, and other factors. In the present study, less than one per cent of the scalps were placed in Type III. Stated in reverse, more than 99 per cent of the scalps could be classified as Types I, II, IV, V, VI, VII, or VIII.

Scalps Which Are Bald, Types IV to VIII. Decision as to the minimal extent of denudation sufficient to constitute baldness was a wholly arbitrary one that was made after a study of scalps in 200 subjects.

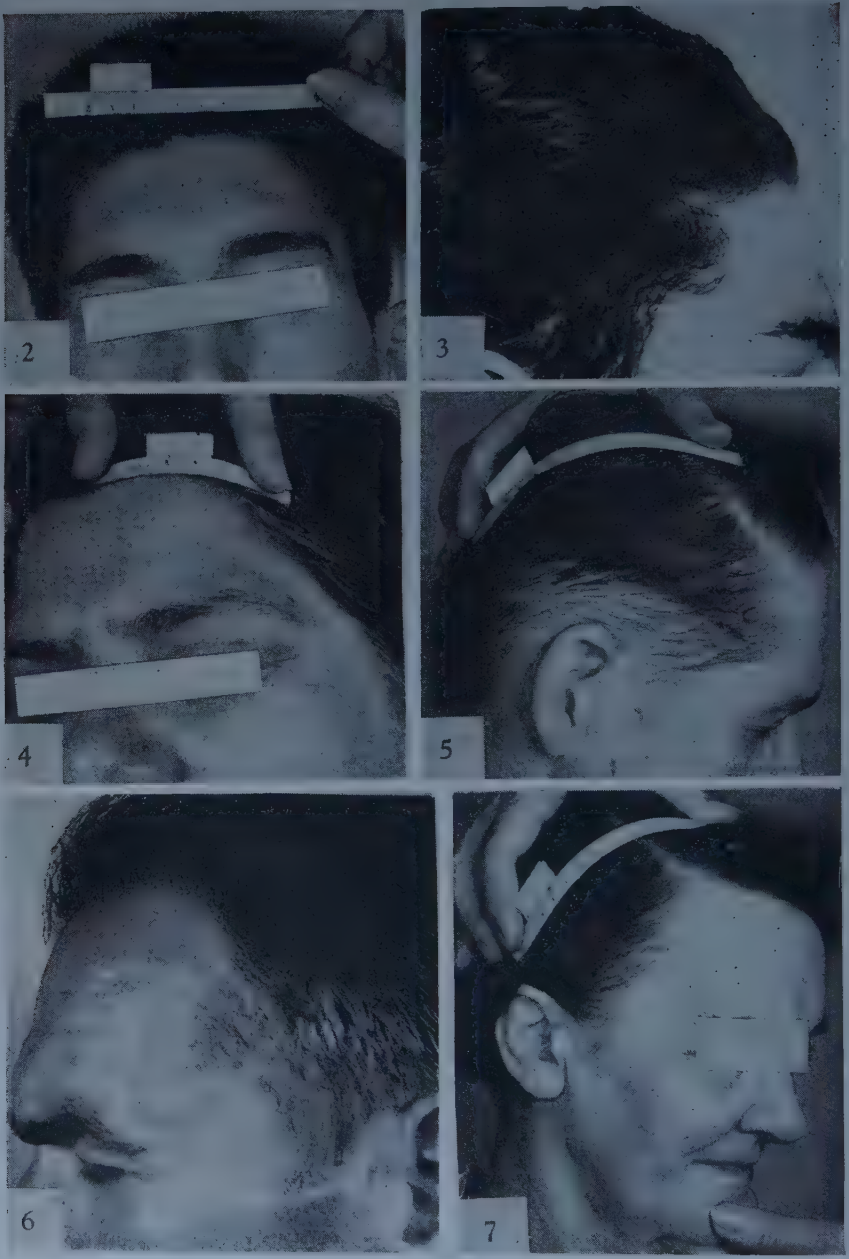
Type IV. This represents the minimal extent of hair loss considered sufficient to represent baldness. Most Type IV scalps have deep frontotemporal recessions which are usually symmetrical and are either bare or very sparsely covered by hair. These recessions extend farther posteriorly than a point which lies 3 cm. anterior to a coronal line drawn between the external auditory meatuses. In most subjects, some hair is also lost along the midfrontal border of the scalp. If hair is sparse or lacking, as a broad band along the entire anterior border of the hair line, the scalp is classified as Type IVA.

In old persons of both sexes, but particularly in men, the minimal amount of sparseness or loss of hair that qualifies as baldness may occur chiefly on the crown. If denudation on the forepart of the head is not more extensive than in Type IV, these scalps with absent or sparse tonsural hairs are classified as Type IV (old).

Type V. With advancing age, men tend to acquire not only extensive frontoparietal and frontal recessions but also a sparseness or absence of hair on the crown. Such extensive but localized areas of denudation on both anterior and postero-superior portions of the scalp, as illustrated in FIGURE 1, are classified as Type V.

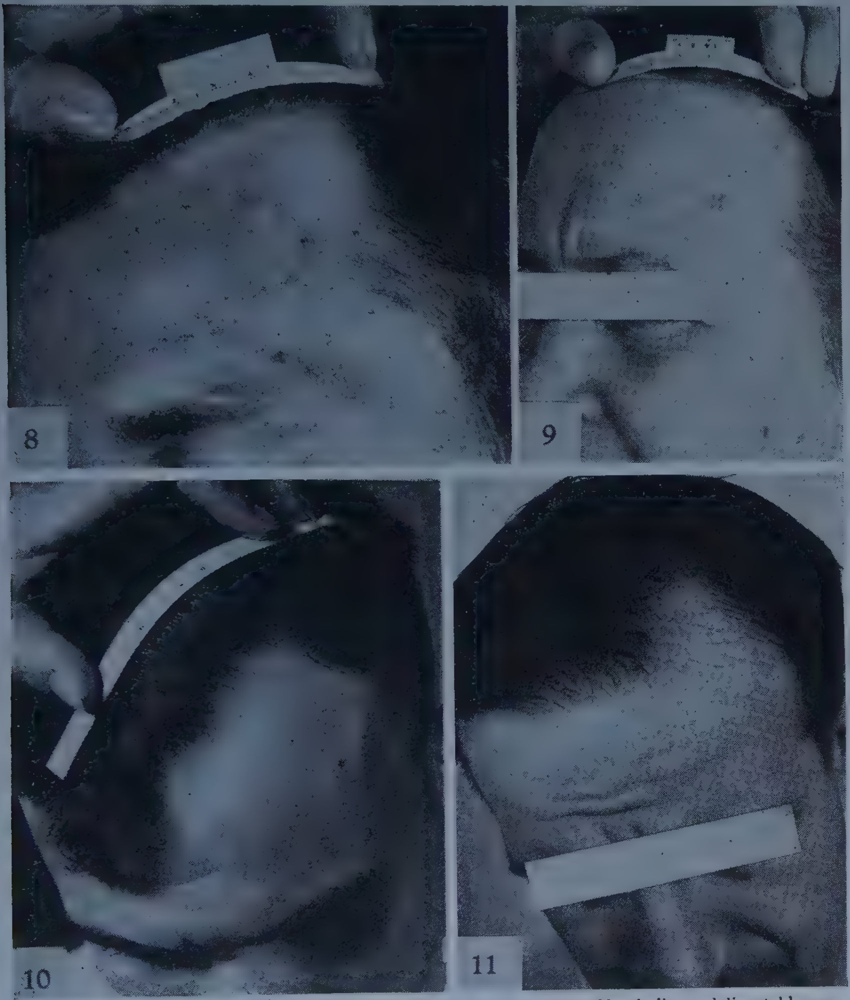
Types VI, VII, and VIII. These types are characterized by areas of alopecia which have the outline of a horseshoe when the head is examined from above. In Type VI, the tonsural region of alopecia remains separated from more anteriorly-located areas of denudation by a laterally-directed bar of scalp in which the hair is only slightly sparse. This bar bridges the region between the fully-haired areas on each side of the head. A peninsula or an island of hair lies in the midline anterior to this laterally-directed hairy bridge. In the variant pattern, Type VIA, the peninsula or island of mid-frontal hair is sparse or lost.

In Types VII and VIII, the horseshoe-shaped area of sparse hair or of



FIGURES 2–7. FIGURES 2, 3, and 4 illustrate Type I scalps in which there is a complete retention of hair. The essential feature of a Type I scalp is the absence of recession of the anterior border of the hair line in the frontotemporal areas. In order of frequency, a Type I scalp is observed in (a) children from one or two years of age until puberty and adults who did not mature sexually (FIGURE 2 shows the scalp of a 30 year-old man who was castrated when 13 years of age), (b) normal women (FIGURE 4 is the scalp of a 41 year-old woman), and (c) normal men (FIGURE 3 is the scalp of a 21 year-old man). FIGURES 5, 6, and 7 illustrate Type II scalps, in which the anterior border of the hair line is indented bilaterally in the frontoparietal regions. Usually, there is also recession to a lesser depth along the entire frontal aspect (FIGURES 6 and 7).

denudation is unbroken by any well-haired, laterally-directed bridge of scalp. This is a result of the spread and confluence of the tonsural and the more anteriorly-located regions of alopecia. The bare area also extends farther laterally and posteriorly than in Type V.



FIGURES 8–11. FIGURES 8 and 9 illustrate Type III, which is comprised of borderline and disputable cases of baldness. FIGURES 10 and 11 exemplify Type IV, which is characterized by deep frontoparietal recessions of the hair line (FIGURE 10), and usually is accompanied by some degree of sparseness or absence of frontal hair (FIGURE 11).

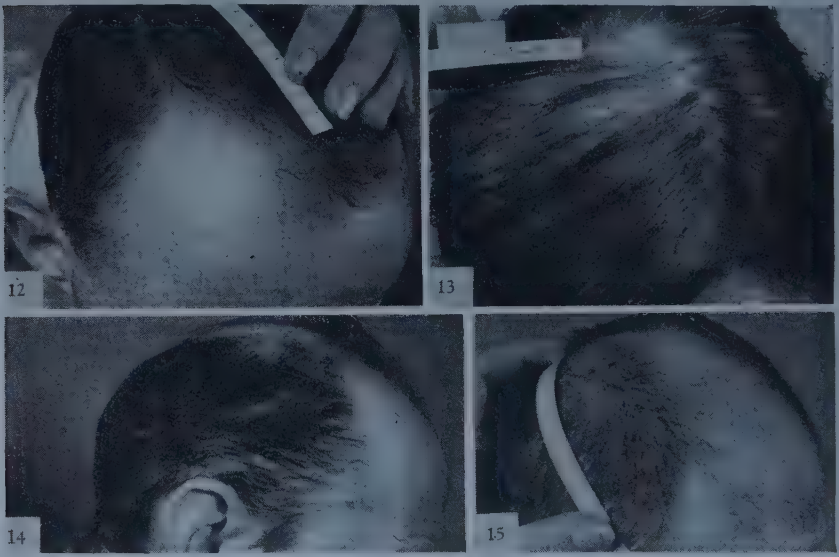
In Type VII, there is still a clump of at least 100 terminal (coarse) hairs in one or more of the three areas shown in FIGURE 1.

In Type VIII, large hairs within the horseshoe-shaped area of alopecia occur only as isolated structures and not in clumps of 100 or more terminal hairs. In most Type VIII scalps, the horseshoe-shaped area is still indented symmetrically on its lateral aspects along a coronal line connecting the external auditory meatuses.

In the most advanced cases of baldness, all clumps of hair may be lost within the horseshoe-shaped area of alopecia. The regions of denudation spread still farther peripherally, both laterally and posteriorly. The hair may also become sparse and thin on the back of the neck, in a semicircle around the ear, and on the anterolateral aspect of the head (FIGURE 1).

Several of these types of scalp are illustrated by photographs of subjects in FIGURES 2 to 15.

Accuracy with Which Scalps Can Be Typed. A method of classification that is based partly upon inspection is open to the criticism accorded subjective procedures. To test the accuracy with which this method can be used, the writer classified the scalps of 200 men and women between 25 and 59 years of age and repeated this process three months later with the same subjects, without reference to notations made at the previous examination. The results of the two classifications were identical in 199 of the 200 subjects. One subject, who had been listed at the first inspection as Type III, or questionably bald, was later classified as Type IV, or definitely bald.



FIGURES 12–15. FIGURE 12 exemplifies Type IV but more hair has been lost in the frontal area than in the man shown in FIGURE 11. FIGURE 13, of a subject with a Type V scalp, shows loss of hair on the tonsure as well as on more anterior aspects of the scalp. FIGURES 14 and 15 are of Type VII scalps, in which the bald area of the head, viewed from above, has a horseshoe-shaped outline but still contains clumps of sparsely-distributed hairs.

To test the accuracy of this method of classification, when used by persons who have not made any special study of baldness, four attendants at a home for the insane were given a set of sketches and photographs like those in FIGURES 1–15 and a brief description of scalp types. After a study period of one-half hour, these 4 attendants carried out separate examinations on an average of 125 of the 200 subjects previously classified by the author. Two per cent of the classifications made by the attendants were at variance with those of the writer. The classifications at variance were (a) Type VI, which was termed Type V, and (b) Type IV, which was tabulated as Type III. Thus, even persons lacking in scientific training and previously unfamiliar with the subject employed the method with 98 per cent accuracy.

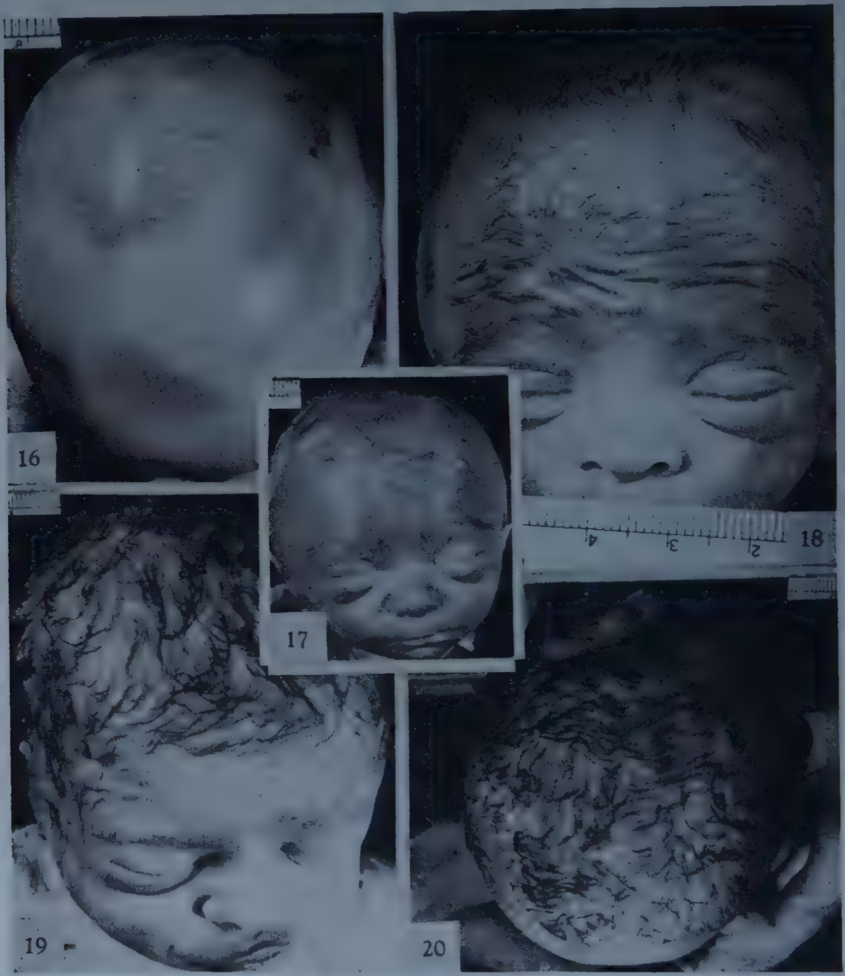
We are engaged at present in counting the number of hairs per unit area, and measuring the size and weight of the hairs. Samples are taken from frontoparietal, tonsural, and supraear regions of the scalp. Although the quantitative analysis of hairs in the various regions is incomplete as yet, it is already obvious that attempts at tedious but quantitative analysis of the piliary status of selected regions of the scalp are less useful in categorization than an inspection which takes the entire scalp into consideration.

Incidence of Scalp Types in Each Sex, Beginning with the Prenatal Appearance of Hair and Extending Until Old Age. Previous reports of the incidence of common baldness, prenatally and postnatally, have been made by Eliot (1892); Sabouraud (1902); Schein (1903); White (1910); Ylppö (1919, 1922); Hrdlicka (1922); Buschke and Gumpert (1926); Stein (1924, 1933); Schwartz and Schwartz (1927); Opitz (1930); and Snyder and Yingling (1935). The conclusions arrived at in previous studies vary widely, as might be expected, since no standards were used, and the extent of alopecia was described generally in terms such as "massive" or "little." In many of the early studies, further errors were introduced both by the use of subjects who chose to consult a dermatologist and by preconceived ideas, such as the theories that baldness is caused by tautness of the scalp, tight-fitting hats, close-cropped hair, seborrhea, or bacteria. In general, the surveys of the incidence of baldness that were based upon hopes of confirming some cherished theory were found to confirm the theory, and are chiefly examples of the fact that one who fails to use yardsticks of measurement can "prove" that which he sets out to prove.

Following this vein of critical evaluation, the part of the present data which applies to the relative percentage of scalp types should also be recognized as incomplete where it applies to individuals 80 or more years of age, since only 22 subjects in the ninth and tenth decades of life were available for study.

(1) *The Prenatal Scalp* (TABLE 1 and FIGURES 16 to 20). Hair was not observed on the surface of the scalp in three of the four youngest fetuses, which had lengths of 141 to 144 mm. from crown to rump (FIGURE 16). Hairs were present on the surface of the scalp of all but one of the fetuses with lengths of 158 mm. or more from crown to rump (TABLE 1).

The first hairs to appear on the surface of the scalp are very fine in texture (FIGURE 17). This hairy coat extends without interruption to cover the forehead. In progressively older fetuses, the hairs of the scalp, but not necessarily those of the forehead, are more coarse (FIGURES 18 and 19). They are grossly uniform in character over the entire scalp until late in prenatal development. Of the 62 fetuses and full-term babies which were examined at the Carnegie Institute of Embryology, eight of these specimens were considered to have reached term. In six of these eight specimens, the hair over the frontoparietal and frontal areas, but not over the tonsure, was thinner than elsewhere on the scalp (FIGURE 20).

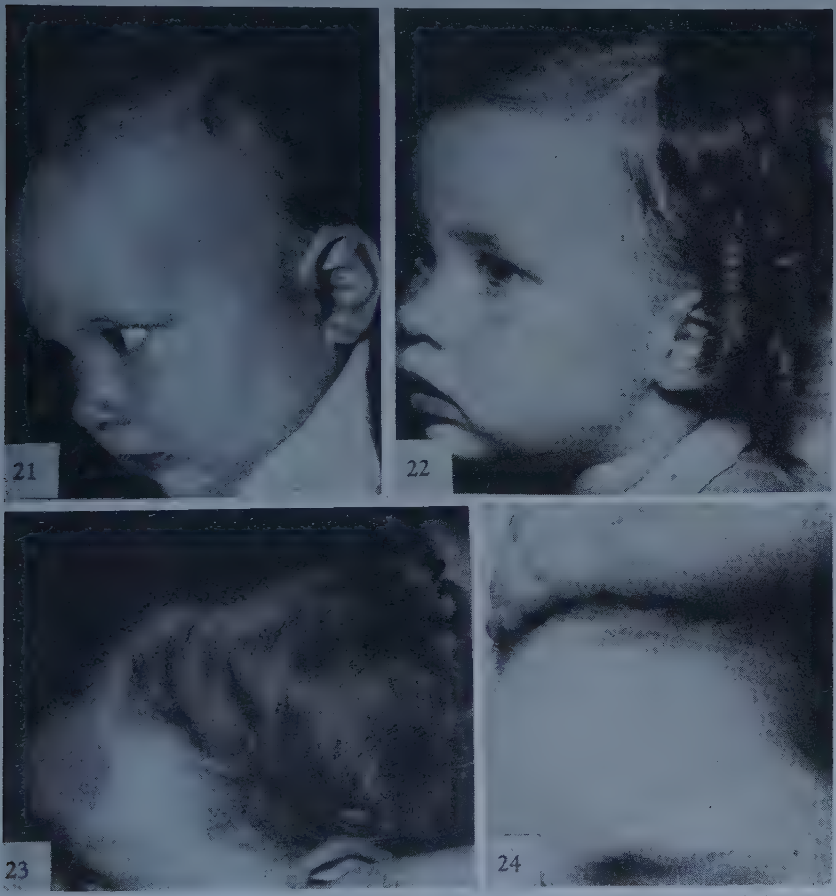


FIGURES 16–20. These show the piliary status in fetuses. Additional data for each of these fetuses are in TABLE 1. FIGURE 16 of fetus 2 illustrates the bare scalp observed in three of the four fetuses, whose scalp length from crown to rump was between 141 to 144 mm. FIGURE 17 (fetus 15) shows the fine texture of the first hairs to appear on the surface of the scalp and also the extension of this hairy coat on the forehead. In older fetuses, hair becomes heavier on the scalp (FIGURE 18 of fetus 25), but not necessarily on the forehead (FIGURE 19 of fetus 50). In specimens considered to have reached full-term, the piliary coat was thinner over frontoparietal and frontal areas than elsewhere (FIGURE 20 of fetus 57).

(2) *The Scalp in the First Two Years of Postnatal Life* (FIGURES 21 to 24). Soon after birth, a marked alopecia develops in many babies in the same areas that become bald in adult men, that is, in the frontoparietal and frontal areas, and even over the tonsure. This process continues for weeks or

months and occurs in both sexes. While hairs are still being lost, new hairs appear on the surface of the scalp and, by the end of the first or second year, most children have a completely hairy scalp of Type I or IA.

(3) *The Scalp from the Second Year of Life until Puberty.* Type I or IA scalps are characteristic of children from the first or second year of life until adolescence, at which time some amount of hair is lost along the anterior border of the scalp in most members of both sexes. Loss of hair tends to be more pronounced in boys than in girls. Therefore, the incidence of scalp types is described separately for each sex, beginning with the middle of the second decade of life.



FIGURES 21–24. Photographs of a girl taken at six months, eighteen months, and at two and nine years of age. The relatively bare areas on the anterior aspect of the scalp in FIGURE 21 represent the maximal change observed in this child after birth. By eighteen months and 2 years of age (FIGURES 22 and 23), the piliary covering of the scalp had become as extensive as it would be in later childhood. FIGURE 24 was taken of the child at 9 years of age.

(4) *The Scalp from the Time of Puberty until Old Age.* In males, baldness (Types IV to VIII inclusive) was observed to become increasingly common

with advancing age until the seventh decade, when the frequency was highest. In females, the incidence did not increase after the fifth decade (FIGURE 25).

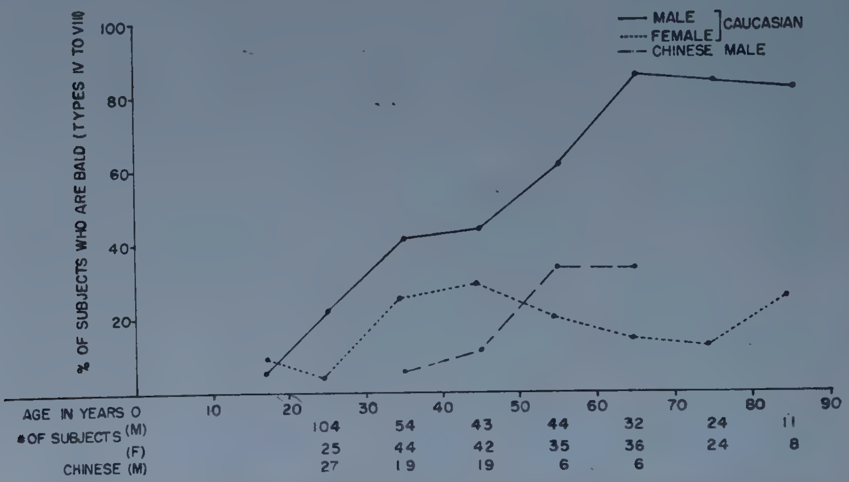


FIGURE 25. The incidence of common baldness (Types IV to VIII) in Caucasian males was found to increase progressively with age and reach a peak in the seventh decade of life. In Caucasian females, this condition was found to be less common and the frequency did not increase after the fifth decade. In Chinese males, baldness was less common than in Caucasian males but a rather similar increase occurred with advance in age. (Based on data for Caucasians in TABLE 2.)

TABLE 2

SCALP TYPES IN CAUCASIANS IN RELATION TO AGE AND SEX

Age in years	*Total no. of subjects*		*Scalp types*																						
			Non-bald								*Bald*														
			I		IA		II		III		IV		IVA		V		VI		VIA		VII		VIII	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
10–14	1						1																	
15–19	18	11	2	2			15	8			1	1												
20–29	104	25	4	3	1	2	69	19	7		17	1			2		3				1			
30–39	55	44	3	4	1	3	27	22	1	4	7	11	1		3		8				3		1	
40–49	43	42		4	1	2	22	24	1		10	12	1				7				1			
50–59	44	35	2	4		2	13	21	2	1	8	7	1		1		4		1		7		5	
60–69	32	36		7	1	3	4	21			2	5	1		3		5		4		10		2	
70–79	24	24		6		3	4	12				3	2		2		2		3		8		3	
80–89	11	8		2			2	4			2	2	2								4		1	
90–99	1	2						1				1	1											
All ages........	333	227	11	32	4	15	157	132	11	5	47	43	9	0	11	0	29	0	8	0	34	0	12	0

It is noteworthy that advanced stages of baldness, Types V to VIII, were not found in any woman, although they were present in 58 per cent of 112 men, 50 to 92 years of age (TABLE 2).

Type I, in which frontoparietal recessions are absent, is one of the most interesting patterns of scalp hairiness. This piliary state characterizes most children from the first or second year of life until adolescence, and was retained in 95 per cent of 20 men who failed to mature sexually (Hamilton, 1942), as exemplified in the subject in FIGURE 2. Type I scalps were lost by all of 11 sexually immature men who were treated for long periods of time with the androgen, testosterone propionate (Hamilton, 1942). Type I scalps were present in only two of 24 virilized women whom the author has had the opportunity to study.

In normal individuals, the disappearance of Type I scalps follows sexual maturation. Maturation is somewhat less likely to abolish a Type I scalp in females than in males. Between 20 and 92 years of age, Type I scalps were present in 21 per cent of the normal women, and in four per cent of the normal men. At all ages after puberty, Type I scalps were more common in females than in males (FIGURE 26).

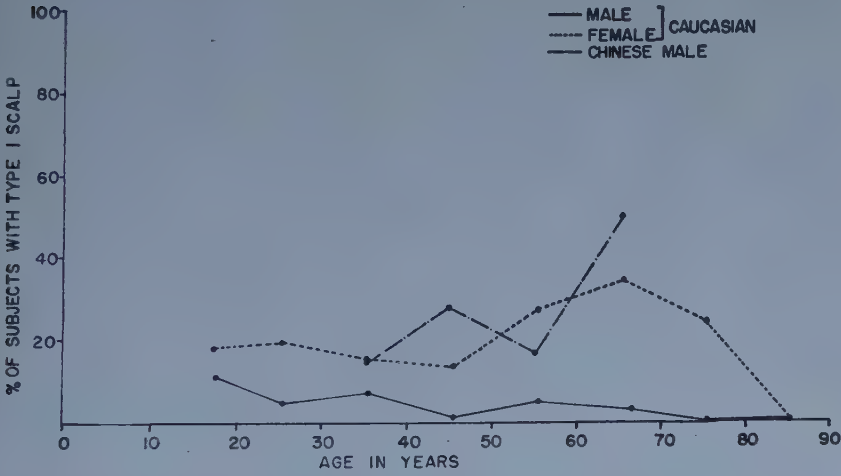


FIGURE 26. Type I scalps, those in which there are no frontoparietal recessions of the anterior borde of the hair line, were retained after sexual maturation by very few Caucasian males, but by a somewha larger percentage of Caucasian females and Chinese males. (Based on data for Caucasians in TABLE 2.)

Type I scalps are retained in some men who have matured sexually and have what appears to be quite normal testicular function thereafter, as judged from physical examination, demonstrated fertility, and their titers of urinary androgens and ketosteroids. Study of the pedigrees of these men indicates that Type I scalps in normal Caucasian men represent familial traits. In normal Caucasian men with Type I scalps, a similar piliary status is much more common in their adult male relatives than among men in the general population.

A tendency to retention of Type I scalps in men seems also to be inherited as a racial trait among Chinese and full-blooded Indians, in whom the condition is more common than among Caucasian men (FIGURE 26). In fact, Type I scalps were found as commonly in Chinese men as in Caucasian women. Quite apparently, then, the capacity for slight degrees of denudation of the scalp in children of Oriental and Caucasian races depends upon a genetic predisposition, just as in the case of susceptibility to development of an extensively bald scalp.

The data in FIGURE 26 show a higher incidence of Type I scalps after the age of the menopause than during the years of reproductive life. This suggests either that mortality rates are low in women with this type of scalp or, more probably, that hair may regrow in the frontotemporal areas of some women after ovarian secretions become reduced.

Almost all Caucasians of both sexes, who do not retain Type I scalps, have acquired at least a Type II scalp within a decade after puberty (FIGURE 26). Type II scalps are the most advanced stage of denudation acquired by most women but are only an intermediate stage in most men (TABLE 2).

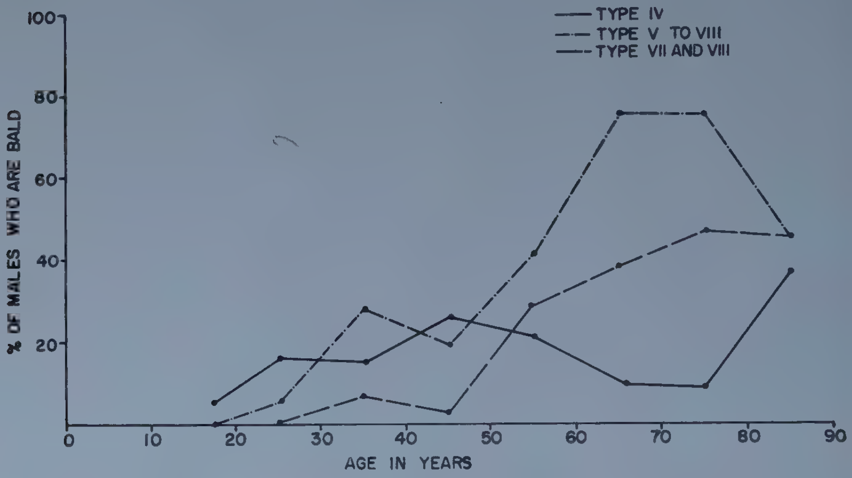


FIGURE 27. The incidence of various degrees of hair loss in Caucasian men at progressive ages. Type IV baldness was found to be an intermediate stage, since most men eventually developed more extensive denudation. The incidence of the most advanced stages of alopecia, Types VII and VIII, continued to increase throughout life. In the ninth decade, only 11 men were available for study; hence further data are necessary before conclusions are drawn with regard to this age. (Based on data in TABLE 2.)

In men, Type IV is, like Type II, an intermediate stage and, in most men, is replaced by more advanced baldness by the seventh decade of life (FIGURE 27). In women, Type IV represents the most advanced stage of alopecia, and the incidence of this type of baldness was found not to increase after the fifth decade of life (FIGURE 25).

More advanced stages of baldness, which form a horseshoe-shaped area and may be referred to collectively as Types V to VIII, increase in incidence in males with age until at least the seventh decade (FIGURE 27).

Comparative Incidence of Common Baldness and Other Forms of Alopecia in Normal Subjects. These series of subjects represent rather unbiased samples of population in that they were selected with regard to range of age and not from individuals who consulted a dermatologist because of the condition of their hair or skin. Since only the common forms of baldness were observed in any of these 312 males and 214 females between 20 and 94 years of age, it is obvious that the common forms of baldness are by far the preponderant kind of alopecia.

Factors Influencing Development of Common Baldness. The first factor is an inherited predisposition which is necessary for development of common baldness or of lesser amounts of denudation. Members of certain races, and of some families among Caucasians, inherit tendencies to retain Type I scalps or to exhibit certain patterns of alopecia.

The influence of inheritance is shown also by the fact that the eunuch whose normal male relatives are not bald does not acquire baldness himself upon treatment with androgens in amounts that are more than adequate to induce baldness in other eunuchs whose normal male relatives tend to be bald (Hamilton, 1942).

Another factor in the induction of baldness is endocrine stimulation, particularly that provided by testes and ovaries. Type II scalps and more advanced stages of denudation, which replace Type I scalps in 96 per cent of normal men after sexual maturation (TABLE 2), failed to appear in males who were castrated prior to puberty and in those who, for other reasons, did not mature sexually (Hamilton, 1942).

Androgens appear to be the agents usually implicated in common baldness, especially in extensive baldness (Types V to VIII), which appears normally only in men. Alopecia does not develop in men who fail to mature sexually but can be induced by androgenic treatment of eunuchs whose pedigrees show them to be susceptible to baldness. Whether denudation to the extent represented by Type IV scalps, which develop in a few women after sexual maturation, is due to androgenic components of ovarian secretions, or their metabolites, is not known at present. Such a possibility exists, however, since (1) androgens have been extracted from ovaries (Parkes, 1937), (2) ovarian secretions can exert androgenic effects at least under unusual conditions (Hill, 1937), and (3) titers of urinary androgens in normal women are two-thirds as much as those in normal men (Gallagher and coworkers, 1937; Dingemanse and associates, 1937).

A third factor is aging. In normal men, advancing age is accompanied by increase in the incidence and extent of baldness. In eunuchs who were castrated prepubertally and given androgenic treatment at a later date, those who are in the second decade of life when treatment is begun tend to lose hair slowly over a period of years, as in most normal young men of their age. In contrast, the eunuch who reaches the sixth decade of life before receiving androgenic treatment loses his hair within a few months of the beginning of treatment. Evidently the susceptibility to alopecia increases with age but is not expressed in the absence of inciting agents like androgens (Hamilton, 1942).

These genetic, endocrine, and aging factors are interdependent. No matter how strong the inherited predisposition to baldness, alopecia will not result if inciting agents, such as androgens, are missing. This is illustrated by the luxuriant scalp hair in old eunuchs who were castrated prior to sexual maturation. Neither are the androgenic inciting agents able to induce baldness in individuals not genetically disposed to baldness. The augmenting action of aging has been referred to above.

The capacity for development of baldness appears to be controlled by factors resident in localized areas of the scalp. Hair tends to be lost first in certain locations. Thereafter, the alopecia spreads to involve neighboring areas somewhat in the fashion that ossification of a cartilage may spread from a center. The manner of this spread of alopecia areas has been discussed elsewhere (Hamilton, 1942), but further evidence is now at hand which strengthens the viewpoint that androgens are but one member of a family of agents capable of inducing loss of hair in those areas of the scalp which are especially susceptible to alopecia. The regions where common baldness occurs in men are the same ones which are most prone to baldness in newborn babies, in individuals who have been subjected to irradiation, as described by Liebow in this monograph,* and in subjects with dietary deficiencies (Chavarría and associates, 1946; Goldman, 1950).

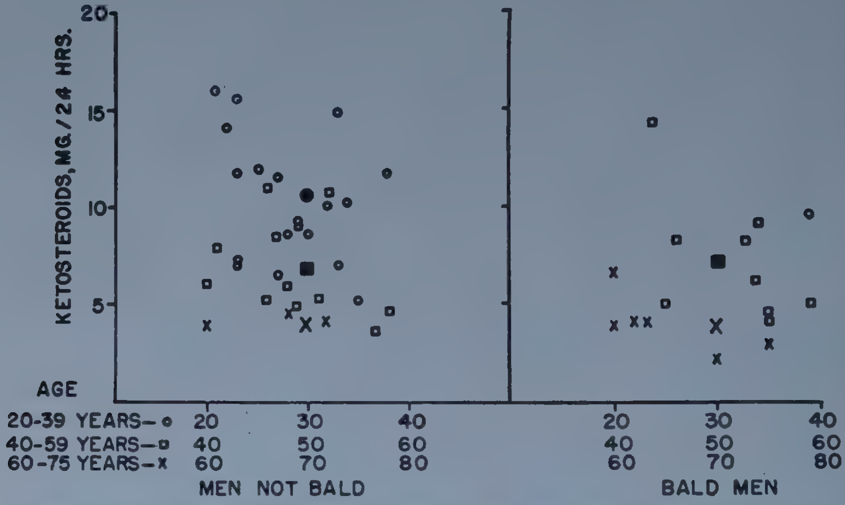


FIGURE 28. Titers of urinary ketosteroids were similar in men, regardless of whether they became bald. Small symbols refer to values for individual subjects, large and black symbols to averages for each age group.

Relationship of the Degree of Androgenic Stimulation to the Extent and Pattern of Common Baldness and to Other Male-Selecting Pathological Conditions. In view of the fact that testicular secretions are the usual inciting agents of common baldness in men, a study has been made of the degree of stimulation provided by sex hormones in bald men as compared with men who are not bald. As tested separately for groups of men in each decade of life, the men who were bald did not differ significantly from those who were not bald with regard to titers of urinary ketosteroids (FIGURE 28) or androgens. Neither did they differ with regard to the extent of growth of a secondary sex character, axillary hair, which can be measured quantitatively (FIGURE 29).

---

* P. 688.

Thus, although androgens induce common baldness, gradations in the degree of baldness among normal men are not accompanied by parallel gradations in the quantity of urinary ketosteroids or in the amount of growth of a secondary sex character, axillary hair, which reflects the degree of stimulation afforded by these steroids.

Instead, there is the paradox that the androgen-dependent condition of common baldness increases in frequency and extent with age, whereas the quantity of these stimulating agents decreases with age (Hamilton and Hamilton, 1948; also unpublished data). The explanation of this paradox is of some importance, since baldness is a condition which may be paralleled to some extent by many of several hundred pathological conditions that select males preferentially and occur with increasing frequency with advance in age (Hamilton, 1948). Pathological conditions of this type contribute materially to the lesser viability of males than of females, which, in the United States, at the present time, amounts to an average of five years' shorter life span in males than in females.

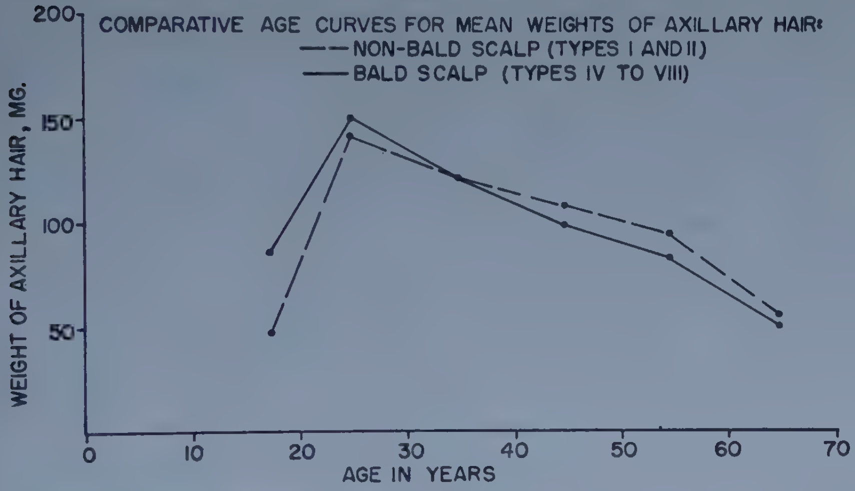


FIGURE 29. Bald men, as compared to men who do not become bald, have similar values for quantitative measurements of a secondary sex character, axillary hair, which reflects the degree of stimulation provided by sex hormones. The number of subjects in each age group was as follows:

Subjects	*Age in years*					
	15–19	20–29	30–39	40–49	50–59	60–69
Bald	3	7	17	14	22	6
Non-bald	11	31	21	16	11	4

This paradox is discussed at length elsewhere (Hamilton, 1948), and the conclusion is drawn that, although the quality of maleness is the basis of this sex difference in viability and in susceptibility to a host of pathological conditions, the role of testicular secretions is but a "setting of the stage, the action on which is controlled by genetic, aging, environmental, and other factors."

Correlation between Development of Alopecia of the Scalp and of the Legs. In men of a similar range of age, 20 to 59 years, the incidence of baldness of the scalp was compared with that of baldness of another area of the body, *i.e.*, the lateral aspects of the legs. A group of 37 men with bald scalps had a slightly higher incidence of alopecia of the legs than did a series of 128 men who were not bald, but the difference was not statistically significant.

Summary

Types or categories of scalp hairiness are described for use as standards in classification and grading of the extent of common baldness. At least 99 per cent of scalps can be classified with reference to these types. The accuracy with which such classifications can be repeated in subsequent independent examinations was 99.5 per cent in surveys by the writer, 98 per cent in surveys by personnel who had no scientific training.

With the use of these categories, the piliary status of the scalp was studied, beginning with fetuses and continuing throughout life until the tenth decade. The bulk of the subjects consisted of 312 males and 214 females, between 20 and 94 years of age.

Hairs on the surface of the scalp were absent in three of four fetuses 141 and 144 mm. in length from crown to rump, but were present in all except one of 58 fetuses 158 mm. or more in length. These early hairs were very fine in texture and extended without interruption to cover the forehead. In progressively older fetuses, the hairs were more coarse on the scalp but not necessarily so on the forehead. The hairs were grossly uniform in character over the entire scalp until near the end of prenatal life. In six of eight specimens considered to have reached term, the frontoparietal and frontal areas, but not the tonsure, were covered by hairs that were less coarse than that elsewhere on the scalp.

Soon after birth, many babies were observed to develop a marked alopecia in the same areas that become bald in adult men, that is, the frontoparietal and frontal regions and, in some babies, the tonsure. Baldness of these areas continued for weeks or months and occurred in both sexes. While hair was still being lost, new hairs appeared on the surface of the scalp until, by the end of the first or second year, most children had a completely hairy scalp of Type I.

Thereafter, a Type I, or completely hairy scalp, was observed to be characteristic of most children until puberty, when hair was lost in members of both sexes.

With advancing age, baldness (Types IV to VIII) occurred with increasing frequency in males until a peak was reached in the seventh decade of life. In females, the incidence did not increase after the fifth decade. Advanced stages of baldness, Types V to VIII, were not found in any women, although they were present in 58 per cent of the 112 men 50 to 92 years of age.

No case of alopecia other than common baldness was observed in any of the 526 subjects between 20 and 92 years of age. Thus, in the general population, the common forms of baldness account for almost all instances of alopecia.

Type I scalps, *i.e.*, those which lack recessions of the anterior regions, and are characteristic of children until adolescence, were retained in 95 per cent of 20 men who failed to mature sexually. This type of scalp was eliminated after androgenic treatment in all of 11 sexually immature men and was present in only two of 24 virilized women.

After normal sexual maturation, Type I scalps were retained in only four per cent of men but in 21 per cent of women. A study of pedigrees in normal men with Type I scalps showed that a tendency in this type of scalp was inherited in certain families among Caucasians and was a racial trait in Chinese.

Baldness was less common and, when present, occurred at a later age in Chinese men than in Caucasian men. The genetic, endocrine, and aging factors, which are responsible for baldness, are interdependent.

Although androgens induce common baldness, the degree of denudation does not parallel the degree of stimulation by steroids, as tested by assays of urinary titers of ketosteroids, or by quantitative measurements of the growth of a secondary sex character, axillary hair, which reflects the extent of stimulation afforded by steroids.

Men with bald scalps had a slightly higher incidence at an early age of baldness in another site, namely on the lateral aspects of the legs, than did men without bald scalps. The difference between the two groups of men, however, was not statistically significant.

References

BLISS, C. I. 1944. Relative potency as applied to the assay of penicillin. Science **100:** 577-578.

BUSCHKE, A. & M. GUMPERT. 1926. Zur Kenntnis des Sexualcharakters des Kopfhaarkleides. Berl. klin. Wchn. **1:** 18–20.

CHAVARRÍA, A., L. GOLDMAN, C. SAÉNZ-HERRERA, & E. CORDERO-CARVAJAL. 1946. Canities and alopecia in children associated with avitaminosis. J. A. M. A. **132:** 570–572.

DINGEMANSE, E., H. BORCHARDT, & E. LAQUEUR. 1937. Capon comb growth-promoting substances ("male hormones") in human urine of males and females of varying ages. Biochem. J. **31:** 500–507.

DOBRINER, K., S. LIEBERMAN, & C. RHOADS. 1948. Studies in steroid metabolism. I. Methods for isolation and quantitative estimation of neutral steroids present in human urine. J. Biol. Chem. **172:** 241–262.

ELLIOT, G. T. 1892. Alopecia praematura: its most frequent cause eczema seborrhoicum. N. York M. J. **57:** 130–136.

ELLIOT, G. T. 1902. The anatomic factor in the production of baldness. J. A. M. A. **38:** 814–815.

GALLAGHER, T. & F. KOCH. 1935. The quantitative assay for the testicular hormone by the comb-growth reaction. Second communication. J. Pharmacol. & Exper. Therap. **55:** 97–117.

GOLDMAN, L. 1950. Personal communication.

HAMILTON, H. B. & J. B. HAMILTON. 1948. Ageing in apparently normal men. I. Urinary titers of ketosteroids and of alpha-hydroxy and beta-hydroxy ketosteroids. J. Clin. Endocrinol. **8:** 433–452.

HAMILTON, J. B. 1942. Male hormone stimulation is prerequisite and an incitant in common baldness. Am. J. Anat. **71:** 451–480.

HAMILTON, J. B. 1948. The role of testicular secretions as indicated by the effects of castration in man and by studies of pathological conditions and the short lifespan associated with maleness. Proc. Laurentian Hormone Conference. **3:** 257–322.

HILL, R. T. 1937. Ovaries secrete male hormone. I. Restoration of the castrate type of seminal vesicle and prostate glands to normal by grafts of ovaries in mice. Endocrinol. **21:** 495–502.

HOLLANDER, F., R. T. FRANK, & E. KLEMPNER. 1941. A dosage response equation for androgen assay by the chick comb method. Proc. Soc. Exp. Biol. & Med. **46:** 1–5.

HRDLICKA, A. 1922. Physical anthropology of the old Americans. I. Pigmentation; grey hair; loss of hair. Am. J. Phys. Anthrop. **5:** 97–143.

LIEBOW, A. 1951. Effects produced on hair by atomic bombs. Ann. N. Y. Acad. Sci. **53**(3): 688.

OPITZ, H. 1930. Ueber Haarausfall bei Neugeborenen und Sauglinger. (Frankfurt a. M.)

PARKES, A. S. 1937. Androgenic activity of ovarian extracts. Nature. **139:** 965.

SABOURAUD, R. 1902. Maladies du Cuir Chevelu. Masson et Cie. Paris.

SCHEIN, M. 1903. Ueber die Enstehung der Glatze. Wien. Klin. Wchnschr. **16:** 611–615.

SCHWARTZ, F. & L. SCHWARTZ. 1927. A csecsemök kopaszságáról. Gyógyászat. **61:** 954–956.

SNYDER, L. & H. YINGLING. 1935. Studies in human inheritance. XII. The application of the gene-frequency method of analysis to sex-influenced factors, with specific reference to baldness. Human Biol. **1:** 608–615.

STEIN, R. O. 1924. Untersuchungen über die Ursache der Glatze. Wien. klin. Wchnschr. **37:** 6–10.

STEIN, R. O. 1933. Die Sauglingsglatze und ihre Beziehung zur Glatze des Erwachsenen. Wien. klin. Wchnschr. **46:** 729–732.

TROTTER, M. 1924. The life cycles of hair in selected regions of the body. Am. J. Phys. Anthrop. **7:** 427–437.

WHITE, C. J. 1910. Alopecia and seborrhea. J. A. M. A. **55:** 1074–1079.

YLPPÖ, A. 1919. Pathologisch-Anatomische Studien bei Frühgeboren. Ztschr. f. Kinderheilkunde. **20:** 212–431.

YLPPÖ, A. 1922. Einige Kapital aus der Pathologie der Frühgeborenen Kinder. Klin. Wchnschr. **1:** 1241–1243.

PATTERNED LOSS OF HAIR IN MAN; PATHOGENESIS AND PROGNOSIS

By Amos E. Light
The Wellcome Research Laboratories, Tuckahoe, New York

Investigations to determine the causes of non-specific baldness are indeed abundant and varied; but the results of such experimentation leave us with little that is factual. That heredity is a contributing factor in hair loss is not to be doubted, although its action may be accomplished indirectly through systemic hormonal relationships as have been previously reviewed.[1]

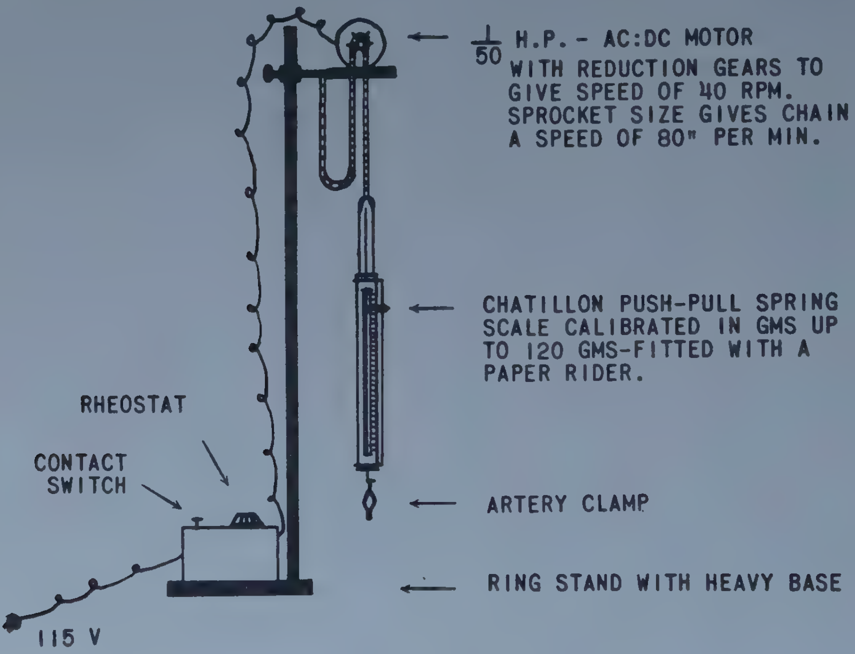
Hormones (especially androgens) are active in hair loss, especially coupled with a predisposing hereditary pattern.[2] Changes in cellular nutrition due to deterioration of the immediate vascular system may be a factor in hair loss. This may be caused by connective tissue changes or by hormonal, nervous, and/or chemical mechanisms. Muscular action may also be involved, as outlined by Szasz and Robertson.[3] Recently, it was learned that there is an increased density of the connective tissue in the area of the hair root in cases of non-specific alopecia.[1] Whether the increase of connective tissue caused the baldness or was only an accompanying feature provides a question for further investigation.

Since non-specific baldness in advanced stages appears to be irreversible, the next logical step in its study would be one of determining at the earliest possible time those individuals who will eventually lose their hair, and then searching for various forms of therapy that would prevent alopecia. The study of the physiological balance of the various sex and cortical hormones involved at various early ages may afford a means of predicting initial tendencies toward baldness, although available evidence indicates that such prognoses appear more to apply to groups of subjects than to individual men.[2] In the scalp itself, periodic determination of various factors, such as temperature, rate of capillary blood flow, scalp mobility, force required to extract individual hairs, and others yet to be found, may reveal this trend.

The method investigated in the following experiments was based on the measurement of the force required to extract a single hair, and was chosen for study because of its convenience and direct approach. It was first described by Basler[4] at Breslau University in 1925. He and his co-workers, especially Otte,[5] have continued research in this field, and several investigators in the United States, including Cronkhite,[6] Au and Bogen,[7] Whitaker,[8] and Copley,[9] have used variations of the original method for various phases of hair research.

Experimentation with various instruments for measuring the amount of pull required to extract a single hair led to development of the apparatus depicted in FIGURE 1. An electric AC-DC motor (1/50–1/100 H.P.) was used for the source of reproducible and constant pull. It was fastened to a ring clamp that could easily be raised or lowered on a heavy ring stand. The motor's speed of 1750 r.p.m. on a 115 V. (A.C.) circuit was reduced by a series of worm gears to a speed of 40 r.p.m. for the emerging shaft. This

$\frac{1}{4}''$ diameter shaft was fitted with a small sprocket which enabled the chain hoist to rise at a speed of 80″ per minute. Final adjustment was made by means of the rheostat. The motor was operated by pressure on the spring contact switch. The spring scale,* graduated in grams up to 120 grams, was suspended from the end of the chain. A paper rider was installed on a steel wire suspended over the gram scale. This rider was pushed along by a short wire extending outwards from the scale pointer and it remained at the maximum value obtained during each pull. Both the chain and rider required resetting at the end of each measurement. A clutch or a reversible motor would facilitate the chain adjustment. An artery clamp fastened to the bottom of the movable part of the scale provided a firm means of attachment to the hair selected for extraction. The apparatus was calibrated with known weights and a zero value was obtained which had to be subtracted from the observed values in order to record absolute grams of pull. The speed of 80″ per minute was chosen because it was the maximum rate at which the observed values would not become exaggerated by the force required to overcome the inertia of the attached weight. A slower motion would yield low values because various cellular attachments of the hair root would not be released at the same time.


1/50 H.P. - AC:DC MOTOR WITH REDUCTION GEARS TO GIVE SPEED OF 40 RPM. SPROCKET SIZE GIVES CHAIN A SPEED OF 80" PER MIN.
CHATILLON PUSH-PULL SPRING SCALE CALIBRATED IN GMS UP TO 120 GMS-FITTED WITH A PAPER RIDER.
RHEOSTAT
CONTACT SWITCH
ARTERY CLAMP
RING STAND WITH HEAVY BASE
115 V

MACHINE FOR EXTRACTING HAIR

FIGURE 1.

---

* Push-pull gauge No. 154-1. Calibrated for vertical pull. John Chatillon & Sons, 85–93 Cliff Street, New York 7, N. Y.

TABLE 1

AVERAGE GRAMS OF PULL REQUIRED TO EXTRACT HAIRS IN AREAS OF MALE SCALPS*

Age	*Subject*	*Supra-eye*	*2 × S.E.†*	*Supra-ear*	*2 × S.E.†*	*Crown*	*2 × S.E.†*	*Test of significance between areas (t values‡)*	
								s. eye–s. ear	*crown–s. ear*
11	JJ	47	± 6.6	48	± 13.3	44	± 16.3	—	—
13	PL	53	11.8	67	11.6	32	14.7	—	3.74
	DS	53	4.2	37	15.9	43	13.1	—	—
14	JP	52	6.3	60	10.2	45	4.5	—	2.68
	CM	46	10.8	58	9.9	51	9.7	—	—
	WE	65	16.8	68	7.7	71	11.8	—	—
	CG	69	7.8	65	9.6	61	8.6	—	—
15	AL	55	10.0	74	7.7	75	18.5	3.00	—
	PK	70	14.5	80	7.2	74	13.0	—	—
	WK	61	7.0	59	11.9	63	8.8	—	—
	JS	26	6.2	29	7.9	31	6.5	—	—
	LB	67	10.9	69	11.6	53	7.8	—	2.29
16	HL	33	6.2	37	7.3	45	8.6	—	—
	AC	52	7.1	63	14.6	66	8.3	—	—
	RG	22	8.5	32	5.3	32	4.6	2.00	—
	RL	65	2.0	76	11.2	68	7.9	—	—
	RH	62	13.4	69	13.8	62	19.9	—	—
	EM	60	10.6	63	13.4	55	10.7	—	—
	HN	78	6.6	83	11.1	81	8.2	—	—
	DM	75	3.9	76	12.6	68	18.3	—	—
	ND	78	11.8	63	11.4	62	7.8	—	—
	PB	36	4.1	37	10.5	44	9.8	—	—
	RW	46	11.8	52	8.1	56	11.6	—	—
17	JK	75	10.9	74	7.3	66	4.4	—	—
	JS	55	12.8	35	12.2	27	4.9	—	—
	RG	56	3.7	48	12.2	54	11.0	—	—
	GL	59	8.4	58	5.0	50	7.7	—	—
	JM	66	12.0	84	11.6	71	7.1	2.14	—
	FB	49	6.3	71	14.9	68	10.0	2.72	—
	HS	51	8.6	52	9.1	57	6.7	—	—
	WH	90	6.9	84	8.9	80	6.1	—	—
	RK	58	11.1	68	16.1	72	7.2	—	—
18	JM	46	2.2	58	6.7	43	12.5	3.43	2.11
	AL	60	11.6	60	10.5	51	10.9	—	—
	OG	76	6.0	67	9.5	59	5.7	—	—
	EW	63	11.9	67	7.0	72	14.7	—	—
	TK	64	11.3	68	12.4	76	12.5	—	—
	AV	59R	9.1	80	7.8	72	6.8	3.50	—
	RP	62	8.4	66	4.3	75	10.5	—	—
	WB	62	9.7	68	14.9	70	7.9	—	—
	CT	59	6.2	62	6.1	54	6.4	—	—
	EH	66	19.8	83	11.3	85	10.7	—	—
	WP	72	14.1	75	12.7	67	5.7	—	—
	AG	61	6.2	61	11.2	45	12.2	—	—
	EW	85	6.6	93	5.0	79	17.1	—	—
	WN	45	9.1	52	6.6	51	11.4	—	—
	HK	89	6.5	92	7.0	81	13.3	—	—
	WR	58	6.5	70	12.2	64	6.4	—	—
	SB	42R	9.1	57	9.1	80	14.8	2.33	—

TABLE 1—(*Continued*)

Age	*Subject*	*Supra-eye*	*2 × S.E.†*	*Supra-ear*	*2 × S.E.†*	*Crown*	*2 × S.E.†*	*Test of significance between areas (t values‡)*	
								s. eye–s. ear	*crown–s. ear*
19	NS	37R	±11.2	59	± 18.3	51	± 13.3	2.06	—
	RO	55	16.3	51	14.9	55	8.5	—	—
	TS	41R	13.6	54	13.9	49	12.2	—	—
	FS	77	11.8	74	15.7	77	11.4	—	—
	RW	65	8.0	68	10.0	73	11.9	—	—
	PE	35R	12.6	53	10.9	56	5.7	2.17	—
	EM	64	7.3	55	9.0	67	7.4	—	—
	HM	48R	12.9	74	8.0	69	5.6	3.42	—
	SK	53	10.9	53	4.1	59	4.7	—	—
	WH	54	13.1	85	6.2	67	16.8	4.31	2.00
	HB	47	7.8	54	10.9	70	8.2	—	—
	WB	46	4.8	61	10.1	54	4.4	2.68	—
	RF	62	6.5	55	10.2	63	13.5	—	—
	GJ	54	7.7	52	6.4	55	4.8	—	—
	MT	44	8.5	49	5.6	63	11.3	—	—
	WB	55	6.0	83	11.5	63	13.9	4.31	2.22
	DB	46	9.9	40	6.0	41	3.8	—	—
	WP	67	10.5	57	10.0	64	6.7	—	—
	PK	44	6.9	58	17.1	57	8.8	—	—
	PK	50	8.6	48	5.5	64	8.3	—	—
	PL	82	10.9	86	8.9	74	14.9	—	—
	WK	63	12.2	84	7.0	83	9.8	2.96	—
	RF	62	9.8	48	12.2	61	6.3	—	—
	JH	40	11.1	44	14.4	50	9.1	—	—
20	PM	41R	6.1	50	15.6	54	7.8	—	—
	PT	81	7.0	69	5.5	82	16.9	—	—
	RD	46R	10.7	60	11.4	60	9.3	—	—
	SL	64	4.5	60	2.9	67	9.8	—	—
	LL	45	7.8	60	16.3	33T	5.6	—	3.13
	WW	28R	7.9	53	14.8	42	15.0	2.98	—
	RS	52	12.1	52	18.6	61	14.0	—	—
	WH	76	8.7	68	17.0	55T	6.0	—	—
	FH	59	15.0	72	9.4	71	12.0	—	—
	AW	45	6.9	61	11.7	57	4.5	2.35	—
	AS	55	5.3	52	10.7	55	7.2	—	—
	GS	67	14.8	90	6.5	76	14.3	2.84	—
21	CE	70	9.1	78	15.5	69	9.8	—	—
	NM	55	10.9	64	4.7	56	8.6	—	—
	RW	53R	5.1	72	8.0	50	6.0	3.96	4.40
	AD	29R	6.0	55	10.0	35	13.4	4.48	2.38
	GM	59	9.1	65	14.0	77	9.1	—	—
23	EW	48	9.2	48	8.4	75	10.3	—	—
24	EL	51R	7.0	69	12.3	69	6.5	2.54	—
	CO	71	6.7	78	9.9	82	11.9	—	—
25	DR	49R	7.5	63	10.9	63	6.2	2.12	—
26	JA	38	8.2	26	8.2	31	8.7	(thinning over ears)	
28	HV	43	5.7	31	9.3	34	10.0	—	—
29	LW	40R	11.0	57	4.8	52	12.4	2.83	—

TABLE 1—(*Concluded*)

Age	*Subject*	*Supra-eye*	*2 × S.E.†*	*Supra-ear*	*2 × S.E.†*	*Crown*	*2 × S.E.†*	*Test of significance between areas (t values)*	
								s. eye–s. ear	*crown–s. ear*
32	JT	43	± 8.0	40	± 10.2	45	± 6.5	—	—
37	ED	45B	7.0	45	6.5	41B	6.1	—	—
39	AL	45B	9.7	54	12.7	31B	7.9	—	3.11
	EL	29B	3.0	38	15.6	21B	9.3	—	—
40	CA	16	5.6	22	4.0	19	3.2	—	—
42	DC	27R	7.8	34	6.7	27	7.3	—	—
43	GM	47R	10.5	57	5.6	41B	13.0	—	2.26
45	NM	60	20.8	69	9.3	64	2.0	—	—
57	RP	42B	11.1	47	10.2	50B	8.2	—	—

* Each line contains data from one subject, and each value represents the average amount of pull in grams required to extract six individual hairs.

† 2 × S.E. — 2 × Standard Error, the number to be added or subtracted from the average value to obtain the upper and lower limits within which 95 per cent of the individual values will be found.

‡ When t equals or exceeds 2.228, the difference is considered highly significant 95 per cent of the time. Only values of 2.00 or over are recorded. (See *Statistical Methods*, G. W. Snedecor, The Iowa State College Press, Ames, Iowa, (1940). Table 3.8, page 58.) R—some hair recession evident; T—thinning of hair; B—Definite sparseness of hair.

TABLE 2

AVERAGE GRAMS OF PULL REQUIRED TO EXTRACT HAIRS IN AREAS OF FEMALE SCALPS

Age	*Subject*	*Supra-eye*	*2 × S.E.*	*Supra-ear*	*2 × S.E.*	*Crown**	*2 × S.E.*
12	MR	45	± 10.9	34	± 14.6	31	± 16.0
22	PG	48	5.1	55	11.5	65	9.6
26	GM	60	5.4	61	11.5	55	9.6
27	SN	28	7.4	34	5.1	24	7.9
29	MP	50	7.8	61	6.3	50	7.7
	MM	32	11.1	38	13.5	45	13.8
30	KA	69	13.4	60	11.4	63	17.0
35	ML	70	9.2	65	13.1	64	15.2
36	AK	51	2.7	41	9.5	49	10.9

* For the females examined, the t values of the supra-ear *vs.* supra-eye and supra-ear *vs.* crown regions did not equal or exceed the highly significant value, which is 2.28.

The measurements were taken in three regions of the scalp: (1) the sides about 1″ superior to each ear; (2) the frontal regions superior to each eye; and (3) the crown. Three hairs were pulled from each side and frontal region and six from the crown, a total of eighteen for each subject. They were selected so as to obtain average types and sizes. The clamp was fastened to the hair about ½″ to 1″ from the scalp and the head of the subject placed so that the pull was perpendicular to the scalp. Unreported preliminary data of over two-years' observations, and that listed in TABLE 1 of 106 males and TABLE 2 of 9 females, all white subjects of many age categories, indicated that the average extraction values were about the same in the three sections of the scalp studied, except in older males who had definite alopecia and in younger males, probably at the age of puberty or older. The values for the eleven-year old male show no significant differences and this is usual for such a young age. It may be seen however, that some older male individuals did have extraction values that were significantly lower in the frontal and crown regions than in the assumed standard side areas. In other individuals, this was not the case. Only average values, which were lower in these two regions than in the region used as the standard, were tested for significance. Areas in which active physiological changes are taking place may be indicated by these differences. It is planned to continue periodic examinations of these and additional subjects in order to determine whether the significant differences exhibited in the frontal or crown regions from the supra-ear area are indicative of later baldness. To date, as shown in TABLE 2, females do not exhibit these significant differences. The measurement of periodic changes in the same regions may also yield useful data.

It was interesting to note that absolute values do not seem to indicate presence or absence of baldness. For instance, the forty-year old male (TABLE 1) had good growth of hair over the entire scalp but low extraction values. It was also found that extremely low values, such as those below 10 grams, were seldom observed, because those hairs so loosely attached had previously been combed or washed from their follicles. Additional data and refinement of this investigative method may probably be obtained by microscopic examination of each hair that is pulled. The force of extraction may then be correlated with the diameter, the age, and the amount of the individual root that is actually pulled from the follicle.

Conclusions

1. The problem of prognosis of non-specific alopecia in males is being investigated by examination of periodic measurements of the amount of force required to extract individual hairs from the crown, frontal, and supra-ear regions of the scalp.
2. In the limited number of subjects studied, significant differences in the amount of force required to extract individual hairs were found between certain scalp regions of some males, but not in any females or males of a presumably prepubertal age.
3. Absolute values could not be correlated with baldness.

References

1. LIGHT, A. E. 1949. Histological study of human scalps exhibiting various degrees of non-specific baldness. J. Invest. Dermat. **13:** 53.
2. HAMILTON, J. B. 1942. Male hormone stimulation is prerequisite and an incitant in common baldness. Amer. J. Anat. **71:** 451.
3. SZASZ, T. S. & A. M. ROBERTSON. 1950. A theory of the pathogenesis of ordinary human baldness. Archiv. Dermat. & Syph. **61:** 34.
4. BASLER, A. 1926. Über die Wurzelfestigkeit der menschlichen Haare. Pflüg. Arch. **212:** 77.
5. OTTE, H. J. 1937. Über die Wurzelfestigkeit menschlichen Kopf-und Körperhaare. Dissertation. Breslau University.
6. DANFORTH, C. H. 1939. Physiology of human hair. Physiol. Rev. **19:** 94.
7. AU, M. H. & E. BOGEN. 1941. Tensile strength of hair and hair roots. J. Lab. & Clin. Med. **27:** 235.
8. WHITAKER, W. L. 1945. "Hair root strength" and the menstrual cycle. J. Invest. Dermat. **6:** 305.
9. COPLEY, A. L. 1947. Pilometric measurements and the rheological properties of hair. Science. **105:** 341.

THE NON-PATTERNED ALOPECIAS

By Carl W. Laymon

Division of Dermatology, University of Minnesota, and Department of Dermatology, Minneapolis General Hospital, Minneapolis, Minnesota

Alopecia, as is well known, is a term which denotes loss of hair. The alopecias have been classified in several ways: for example, congenital, premature, or senile. They have also been divided into the classifications: patchy, diffuse, temporary, and permanent. Alopecia may be associated with atrophy and/or scarring, as is the case in burns, scalds, lupus erythematosus, pseudopelade, trauma, epithelioma, pyodermas (furuncle, carbuncle, ecthyma, acne necrotica), fungous infections (favus, kerion, and other forms), syphilis (chancre, ulcero-nodular syphilid, gumma), tuberculosis (lupus vulgaris, verrucous tuberculosis, scrofuloderma), dermatitis herpetiformis, perifolliculitis abscedens et suffodiens, acne keloidalis, and gangrenous herpes zoster. Alopecia may also be associated with inflammatory diseases of the scalp, such as fungous infections which do not lead to scarring, and seborrheic dermatitis. In the following other cases, there may be no obvious changes in the scalp: alopecia areata, congenital alopecia, symptomatic alopecia, tricholtillomania, and ordinary male baldness.

Alopecias also may be patterned or non-patterned. Except for ordinary male baldness and the symptomatic alopecias, practically all other types are of the non-patterned variety. Symptomatic alopecia is a loose term for hair loss occurring during acute illnesses, such as scarlet fever, typhoid and influenza, pregnancy, post-operative crises, and tuberculosis, and accompanying exfoliative dermatitis due to drugs or other causes. Almost without exception, these cases of alopecia are diffuse, and therefore patterned. In ordinary male baldness, the hair usually disappears from the fronto-temporal region, either alone or in conjunction with thinning at the vertex, thus forming a patterned type of alopecia.

Practically all dermatoses involving the scalp which lead to scarring or atrophy produce a non-patterned type of alopecia. Most of these disorders, however, do not cause much diagnostic confusion, since they can usually be recognized by their clinical features, plus appropriate laboratory procedure when indicated.

The most important diseases characterized by permanent non-patterned alopecia, which sometimes cause considerable diagnostic difficulty, are pseudopelade, folliculitis decalvans, and lupus erythematosus. Because of time limitations, these are the disorders which will be discussed in this presentation, although alopecia areata will be considered also. It is relatively common and occasionally may be confused with the other disorders, especially pseudopelade and folliculitis decalvans.

Alopecia Areata. Alopecia areata is characterized by sudden loss of hair occurring in one or more circumscribed patches and usually involving the scalp, although the beard, genitalia, axillas, brows, eyelids, and general surface of the body may be affected. The term alopecia totalis is applied to loss of hair over the entire scalp, while the term alopecia universalis is used to denote those rare cases in which all of the hair on the body is lost.

Occasionally, vague peresthesias or neuralgias may precede the hair loss. In some cases, the loss of hair is gradual and, instead of one area, there may be several which develop simultaneously or at varying intervals. The patches, if multiple, are often of various sizes, from the size of a dime to that of a silver dollar. They are usually round or oval, and their surfaces smooth and shiny. The fact that there is no visible evidence of inflammation is of diagnostic importance. Broken hairs, termed "exclamation point hairs" may frequently be seen, especially at the periphery of the bald areas. The cause is vague.

The histology of alopecia areata has been studied by many investigators and, in general, their findings agree. The one striking epidermal change is the lack of pigment. This was noted by Laymon and Murphy,[1] even in microscopic sections from a Japanese girl who had alopecia areata for only a week. In the papillary portion of the cutis, there are usually a few small collections of lymphocytes. Deeper in the cutis, the infiltration is usually greater and is most intense about the vessels, sebaceous glands, and follicles. The double rows of hairs in the follicles, which were described by Sabouraud, were not seen in any of the cases which were studied; in fact, there were practically no hairs found. Sprouting or budding of the follicles was also absent. Sebaceous and sweat glands appeared normal, rather than atrophic, as noted by some observers. No sclerosis of the connective tissues was found. In sections from cases of one or two year's duration, the only noteworthy difference was the subsidence of the inflammatory reaction.

Pseudopelade. Pseudopelade begins insidiously, without visible signs of inflammation, and pustular folliculitis is completely absent throughout its entire course. It is usually asymptomatic. In most cases, there are multiple small areas, which average from a few millimeters to a centimeter in size. Although the vertex is usually involved first, the disease may begin on the posterior or temporal portions, and, although it is a rare occurrence, lesions may appear simultaneously all over the scalp. The color of the involved skin is usually white or ivory, although occasionally it may be pink. The skin in the affected areas is smooth, soft, slightly depressed, and resembles the peel of an onion. Its sensitivity is reduced in most cases. The lesions are round or oval and frequently tend to coalesce, to form large plaques having irregular contours. In some cases, there are a few large plaques with multiple small bald areas around them. The largest plaques may be the size of the palm and usually present irregular sinuous or polycyclic shapes. The number of plaques is extremely variable but is usually large. Despite this, the scalp never becomes entirely bald, and some healthy hair remains, frequently in tufts, scattered at various points over the surface of the scalp. The lesions are sharply demarcated, and there are no broken, lanugo, or exclamation point hairs. When the hairs are pulled, they bring the lining of the follicle with them and are surrounded by a succulent, glassy sheath, which resembles a favic hair.

The course of pseudopelade is extremely slow. Although, in some cases, it is constantly progressive, most often it is characterized by periods of quiescence and activity of varying duration. In most cases, the patient seeks advice only when the disease has been present for many years. Pseudopelade usually requires ten or more years before much alopecia appears.

Nothing definite is known concerning the cause of pseudopelade, although most observers favor the theory that it represents a low grade follicular infection. Cultures and direct examinations of hairs have given no definite information. The disease usually appears in males from 20 to 45 years of age and there is no evidence that it is contagious.

A prominent feature of pseudopelade, histologically, is the occasional thinning of the epidermis to the point of three layers of cells. In some cases, the rete pegs are entirely flat. Perhaps the most striking feature is the total disappearance of follicles and sebaceous glands. Next in importance are the perpendicular columns of fibrous tissue which replace the follicles. The third feature is the tendency to sclerosis of the cutis down to the level of the sebaceous glands. In most cases, however, there is not complete homogenization of the connective tissue. In most instances, the sweat glands and arrector muscles are preserved, although there may be cystic dilatation of the former. Plugging, by horny material, of the remnants of follicular openings has been described by some observers and found to be absent by others. The inflammatory changes in the cutis are small collections of lymphocytes distributed chiefly about the blood vessels and sclerotic remnants of the follicles. Sections stained by the Weigert method show absence of elastic fibers at the sites of the cicatrized follicles.

Folliculitis Decalvans. Folliculitis decalvans is a rare follicular inflammatory process which eventually leads to circumscribed patches of cicatricial baldness. Through progressive involvement of neighboring follicles, the plaques gradually increase in size from .25 to two or three centimeters. They are usually round or oval in shape and, because the symptoms are slight, the disease is usually present for several months before the patient seeks advice from a physician. The affected, bald, atrophic areas are usually white or yellowish-white and, on close examination, inflammatory papules or pin-head-sized pustules can be noted at the border, the presence of which is essential for the diagnosis. As the active lesions involute, superficial crusts remain, and, when the affected hair is shed, a tiny red spot remains, the color of which gradually fades. The follicle is completely destroyed and is replaced by fibrous tissue, and, since the center of the lesions represents the point of oldest involvement, it appears as a smooth, shiny, bald scar with complete obliteration of the follicular openings. The disease progresses slowly and, even during active phases, leads only to slight itching.

In some cases, an area as large as the palm may eventually be cleared of hair. The following characteristics have been considered of most diagnostic importance in folliculitis decalvans: (1) inflammatory changes around the follicles of the scalp in the beginning; (2) peripheral extension and central healing leading to the patchy cicatricial alopecia due to chronic inflammatory infiltration in the perifollicular connective tissue; (3) the almost complete lack of symptoms; (4) the chronic course; and (5) the extraordinary resistance to treatment with resultant unfavorable prognosis.

The cause of the disease is unknown, although the consensus is that folliculitis decalvans is a bacterial infection. In 1888, Quinquaud[2] found various cocci which did not lead to death when inoculated in rats, mice, and rabbits, but which produced folliculitis in rats, rabbits, and humans when applied with friction. The findings of other workers have been indefinite and inconsistent. McCarthy[3] regards folliculitis decalvans as a disease of the scalp, analogous to an attenuated form of sycosis with a microbian involvement of the bottom of the follicle and the hair papilla.

Laymon and Murphy studied two cases of folliculitis decalvans histologically. In each, two biopsies were performed, one from the center of an atrophic, hairless plaque, and a second from an area of the lesion toward the border, which contained a pustule. The histopathologic findings in the central portions of the plaque seemed identical in all respects to those of pseudopelade. In the sections containing pustules, all of the features of pseudopelade were seen, plus abscess formation just below the basal layer and occupying most of the upper half of the cutis. The abscesses contained a preponderance of polymorphonuclear leucocytes as well as a considerable number of lymphocytes. In the two cases of folliculitis decalvans, the foci of infiltrate throughout the cutis seemed larger and more numerous than in the cases of pseudopelade, although the differences were not great enough to be of diagnostic importance.

Lupus Erythematosus. Lupus erythematosus of the scalp begins with small, wine-red, non-diagnostic flecks around the follicles, which gradually enlarge and coalesce to form irregular plaques. On various parts of the scalp, these lesions may remain superficial and erythematous, but in most cases their centers become slightly depressed, atrophic, and lighter in color, while the prominent borders remain bright red. The plaques often become covered with white or grayish-white scales, the removal of which results in slight bleeding. In most cases, there are conical plugs in the dilated follicles. Telangiectatic vessels may be seen both at the borders and in the centers of the plaques. As the cicatrizing process continues, small, white, bald areas appear, and the older the lesion, the less hair remains. In general, the plaques are completely free of hair in the centers, while a few hairs often remain at the hyperkeratotic borders. In certain erythematous lesions without atrophy, the hair may not be lost, but in these cases there is no confusion with pseudopelade or folliculitis decalvans.

Lesions of lupus erythematosus may appear on any part of the scalp. There may be one large plaque or many small ones. The disease is more frequent in females than males. Galewsky[4] stated that, when the disease begins on the face, 20 per cent of men have scalp-lesions as compared to the figure of 50 per cent in women. Infrequently, lupus erythematosus begins on the scalp, but remains limited to that location only rarely.

Michelson[5] brought out the fact that the pathologic changes of chronic lupus erythematosus are made up of three distinct processes: first, a vascular or perivascular disturbance; second, edema and subsequent damage of connective tissue, followed by an infiltration; and third, epidermal and follicular alterations. He emphasized that the changes depended, to some degree, on the type of lesion under consideration, and that a uniform picture for lupus erythematosus does not exist. Even though the epidermal changes are secondary to the inflammatory process in the cutis, they are of aid in diagnosis.

The author[6] recently made a histological study of 15 cases of discoid lupus erythematosus of the scalp. The degree of hyperkeratosis was mild and insignificant in some, moderate in others, and extreme in still others. Dilatation and plugging of the follicles was present in some cases but was not a constant finding. The granular layer was preserved in almost every instance and, in many cases, was thickened. Epidermal atrophy was present in almost all cases, although, in a few, there were areas where papillomatosis was noted. In most cases, the basal outline was almost flat and the basal layer was vacuolated, degenerated, and disrupted.

In the cutis, dilatation of the small blood vessels, particularly in the upper portion, was a prominent feature. Edema, which varied from moderate to intense, was more severe as a rule than in any of the diseases previously discussed. The infiltrate was present in various amounts and in masses of different shapes. Some were globular, apparently being separated from each other by practically normal connective tissue. In some sections, the infiltration was follicular and perivascular, although at many points it was neither. The lymphocyte was the predominant cell, although a few leucocytes were seen. Plasma, mast, and giant cells were conspicuous by their absence. Deeper in the cutis, the infiltrate was rather heavily focused about sweat glands. In most of the cases of lupus erythematosus of the scalp, the sebaceous glands and follicles are destroyed early by the inflammatory process. The elastic tissue was severely disrupted in the papillary part of the cutis. The infiltrate in lupus erythematosus was distributed in an entirely different manner in active cases than in other types of cicatricial alopecias, such as pseudopelade and folliculitis decalvans, being much heavier and more diffuse in the upper part of the edematous cutis in lupus erythematosus.

Sections from old inactive lesions of lupus erythematosus were entirely non-diagnostic. The epidermis was extremely atrophic, the rete pegs completely flattened, the entire cutis practically homogenized, the sebaceous glands and follicles gone, and the sweat glands so shrunken that they, too, were almost gone. The infiltrate, perhaps the chief feature in the active process, had entirely disappeared.

Differential Diagnosis of the Non-Patterned Alopecias. Pseudopelade differs greatly from alopecia areata by the multiplicity and the smallness of the points of attack, by the irregularity of the border of the plaques, by the presence of a glassy sheath about the epilated hairs, by the absence of exclamation point hairs, and by the termination of the disease in permanent baldness. According to all observers, the presence of follicular inflammatory papules and pustules is essential for a diagnosis of folliculitis decalvans, a feature which is completely absent in pseudopelade. If these are absent (as in some cases of long duration), a differentiation from the latter is impossible. Active lesions of lupus erythematosus of the scalp are always characterized by redness and scaling, which, along with dilatation and plugging of the follicles, permits differentiation from other non-patterned alopecias.

The presence of concomitant lesions on the glabrous skin is an additional diagnostic aid. In old "burned out" lesions of lupus erythematosus of the scalp, in which there is only scarring, clinical differentiation from pseudopelade or folliculitis decalvans may be entirely impossible. Inactive favus, with the presence of lusterless, dull, gray, dry, broken hairs, and scutula, plus the demonstration of fungi, permits positive diagnosis from other forms of nonpatterned alopecia. Circumscribed scleroderma of the scalp may superficially resemble pseudopelade, although palpation permits absolute differentiation, since the lesions of the former are hard in consistency. In certain rare cases of severe seborrheic eczema, repeated attacks of folliculitis may produce a picture which simulates folliculitis decalvans. Concomitant findings of seborrheic eczema on other parts of the body expedite a correct diagnosis.

Summary

(1) With the exception of ordinary male baldness and the symptomatic alopecias, most all other forms of alopecia are of the non-patterned variety. Alopecia can be produced by a large number of dermatoses which lead to scarring, most of which do not cause much diagnostic confusion, since they can usually be recognized by their clinical features, plus appropriate laboratory procedures when indicated.

(2) A few non-patterned types of alopecia, such as pseudopelade, folliculitis decalvans, and lupus erythematosus, may cause considerable diagnostic difficulty.

(3) Alopecia areata of a few weeks' to a months' duration, shows moderate inflammatory changes histologically. Follicles and glands are normal. No sclerosis is seen. The epidermis is normal.

(4) Histologically, pseudopelade and folliculitis decalvans show a high degree of epidermal atrophy, sclerosis of the cutis, and perpendicular columns of fibrous tissue replacing the follicles. Sebaceous glands are obliterated, although the arrector muscles and sweat glands are preserved. The latter, however, are occasionally cystic. Inflammatory changes are not intense, much less so than in lupus erythematosus.

(5) Chronic discoid erythematosus of the scalp presents changes which permit differentiation from pseudopelade and folliculitis decalvans. These changes consist of vascular dilatation and edema in the cutis, with secondary epidermal changes, such as hyperkeratosis, atrophy, follicular dilatation, and disruption of the basal layer. Old inactive lesions of lupus erythematosus of the scalp are non-diagnostic.

(6) In a previous report, Laymon and Murphy[1] made a histologic study of six cases of ordinary male baldness. The findings in all were about the same. The patients ranged in age from 48 to 80 years. There were no outstanding epidermal changes, except slight atrophy, which was not enough to be of any consequence. The sebaceous glands were numerous and large, in all sections which were examined. The sweat glands were plentiful in

most sections and seemed normal. Since, in all but one case, the patients had been completely bald for 15 to 30 years, many follicles had apparently disappeared and most of those observed were atrophic. Inflammatory changes were minimal and of no diagnostic importance. In sections stained by the van Gieson method, the collagen appeared normal, and the sections stained by the Weigert method showed no disturbance of the elastic tissues.

References

1. LAYMON, C. W. & R. J. MURPHY. 1947. The cicatricial alopecias. J. Invest. Dermat. **8:** 99–122.
2. QUINQUAUD, E. 1888. Folliculite destructive des régions velues. Bull. et mém. de la soc. des Hôp. de Paris **5:** 395–398.
3. MCCARTHY, L. 1940. Diseases of the Hair. C. V. Mosby. St. Louis.
4. GALEWSKY, E. 1932. Erkrankungen der Haare and des Haarbodens, Handbuch der Haut—u. Geschlechtrskrkh. **13**(1): 129–437. Julius Springer. Berlin.
5. MICHELSON, H. E. 1949. The problem of lupus erythematosus. Calif. Med. **70:** 1–5.
6. LAYMON, C. W. A histologic study of some scaly dermatoses involving the scalp. Arch. Dermat. & Syph. In press.

HIRSUTISM

By Grosvenor W. Bissell
Department of Medicine, University of Buffalo School of Medicine and the Endocrine Clinic, Edward J. Meyer Memorial Hospital, Buffalo, New York

By definition, hirsutism is abnormal hair growth, particularly in the female. Since, by definition, the female is specifically implicated and since, clinically, hirsutism is seldom if ever a problem in the male,* we shall confine this discussion to hirsutism in women.

It is somewhat surprising to learn that about 30 per cent of women have heterosexual hypertrichosis of some degree.[1] To some it is a matter of little concern, but, in the more severe cases, the "bearded lady" becomes an important entity: to the showman a source of profit, to the layman an object of amusement, to the physician a riddle in diagnosis, to herself an object of ridicule, social rejection, and abject misery, which, at times, has resulted in suicide.

Permanent hirsutism may accompany a number of organic diseases. The most common of these are dysfunctions of the adrenal cortex, anterior pituitary gland, and ovary.

The Adrenal Cortex. The pathological lesions of the adrenal cortex associated with hirsutism may be hyperplasia, an adrenal cortical carcinoma, or functioning adrenal cortical adenoma. Clinically, two rather distinct syndromes, may result: the Cushing syndrome or the adrenogenital syndrome.

Cushing's syndrome has the following clinical characteristics. There is facial hirsutism, associated with a male pubic hair distribution and usually some increase in body hair. The skin is thin and acne is common. The face is broad and moon-shaped, rubicund in appearance. There is obesity of central distribution, with purplish abdominal striae and a buffalo hump of fat over the posterior cervical region. The clitoris is somewhat hypertrophied and amenorrhea or oligomenorrhea is common. Hypertension is constant. Roentgenographic studies reveal osteoporosis of the spine and pelvis. There is an associated polycythemia with lymphopenia and eosinopenia. A mild type of insulin-resistant diabetes is characteristic. The urinary excretion of creatinine is diminished, and there is marked increase in the urinary output of 11-oxysteroids, with normal or moderately increased 17-ketosteroid excretion.

It is widely held that Cushing's syndrome represents a form of hyperadrenocorticism in which there is an overproduction of those adrenal hormones having to do predominantly with carbohydrate metabolism, the so-called "S-hormones" of Albright.[2]

The adrenogenital syndrome is associated with facial hirsutism, male distribution of pubic hair, and, often, a marked increase in body hair. The status of the skeletal musculature is excellent and the general body configuration is male. Clitoral hypertrophy is often marked and amenorrhea is present. Acne is common and the voice is deep. The output of 17-ketosteroids is increased. Classically, there is no tendency to diabetes, hypertension, polycythemia, or the excretion of excessive amounts of corticoid substances in the urine. This is a clinical syndrome associated with an increased output of adrenal androgenic steroids, or "N-hormones" of Albright.[2] If this syndrome is initiated in early uterine life, pseudo-hermaphroditism results.

---

* A possible exception to this statement is that of the so-called "dog man," a rare entity with some degree of familial occurrence, affecting both males and females, in which heavy vellus hair covers the entire body including the face. The famous Jefticheff and Schwe Maong families are examples.[1]

If either of these syndromes is associated with an adrenal cancer, the output of β-ketosteroids in the urine is markedly increased. Although these two syndromes may be quite distinct, instances occur in which there is a commingling and overlapping of symptoms.

The Anterior Pituitary. Basophilic tumors of the anterior lobe may give rise to a clinical state indistinguishable from the hyperadrenocorticism of the Cushing syndrome. When such a tumor is present, the entity is referred to as Cushing's disease, in contradistinction to the clinical picture produced when only the adrenal is implicated. In Cushing's disease, however, the adrenals are almost always hypertrophied.

Eosinophilic tumors of the pituitary cause acromegaly, which is often associated with hirsutes, especially of the body. Although facial hirsutism may occur,[3] it is unusual. Diabetes and hypermetabolism are common accompaniments of the marked skeletal overgrowth, soft tissue hypertrophy, and visceromegaly.

The Ovary. Tumors of the ovary, especially arrhenoblastomata, are rare but are important causes of hirsutism. They are invariably associated with amenorrhea, or severe oligomenorrhea, associated with marked virilization, but there is no evidence of other metabolic involvement. The urinary 17-ketosteroid excretion is within the normal range,[4] although occasional moderate elevations have been reported.[5]

Miscellaneous. Hirsutism has been observed in association with thymic tumors (with secondary adrenal hyperplasia,[6, 7] dermatomyositis,[8] concussion of the brain,[9] lutein cell tumors,[10] diffuse ovarian luteinization,[11] teratomata, neuritis of several varieties, mumps, encephalitis, mental defection, and multiple sclerosis.[12] Recently, Shroeder has described a group of women with hypertension, rapidly appearing central obesity, irregular menses, low sweat sodium excretion, and marked sensitivity to the pressor effects of desoxycorticosterone, whose hypertension and obesity are responsive to low sodium diets. Such cases have been referred to as "pseudo-Cushing's syndrome." About one-sixth of these cases display hirsutism of some degree.[13]

Idiopathic Hirsutism. By far the commonest type of hirsutism is so-called idiopathic or essential hirsutism (FIGURE 1). Although there is quite a formidable amount of literature referring to the hirsute syndromes associated with the somewhat rare organic causes mentioned previously, information regarding this form of hypertrichosis is meager. For that reason, we have studied this type of hirsutism in some detail.[3] Idiopathic hirsutism occurs most commonly at puberty, during or immediately after pregnancy, or past the menopause. There is often a strong family history of hirsutes and the entity is commonly seen in some races more than others, notably Southern Europeans, Syrians, and Jews.

Many of our patients shaved daily, and the majority displayed a male pattern of pubic hair distribution. The general body hair was often but not always increased. Whorls of hair were often seen with hypertrichosis of the anterior chest and back, and circumareolar hair occurred in a small percentage of cases. In approximately 50 per cent of our patients, there was some degree of menstrual abnormality. In a similar percentage, the clitoris, if not hypertrophied, seemed to be the largest size consistent with normality. The labia majora were characteristically hypertrophied and pouting in appearance, and the pubic hair covering them was profuse. Occasionally, we have found an associated infantile uterus, but this finding does not seem frequent enough to be significant.

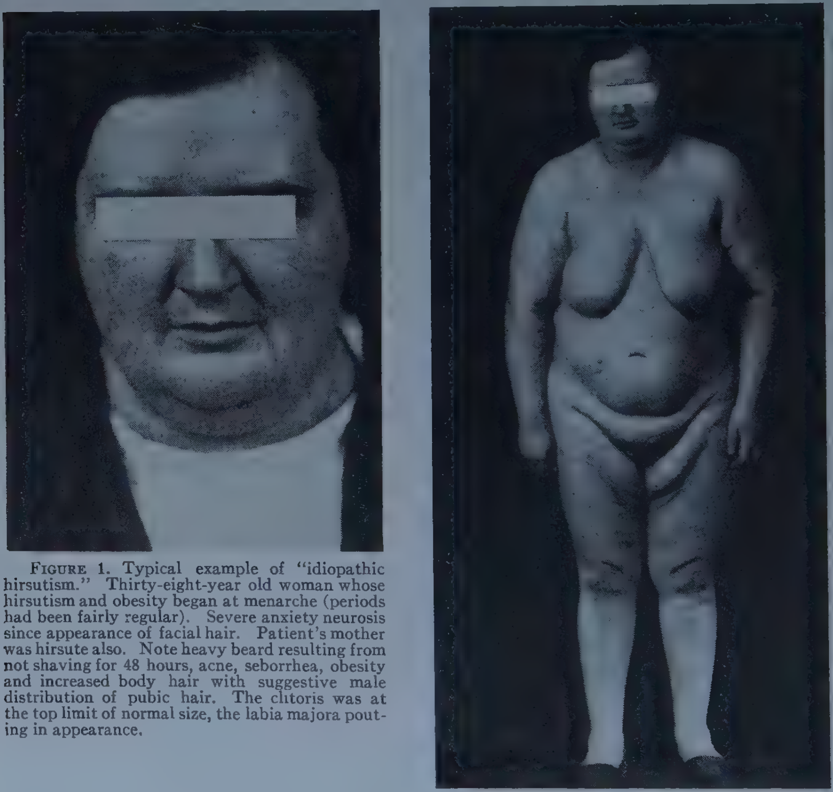


FIGURE 1. Typical example of "idiopathic hirsutism." Thirty-eight-year old woman whose hirsutism and obesity began at menarche (periods had been fairly regular). Severe anxiety neurosis since appearance of facial hair. Patient's mother was hirsute also. Note heavy beard resulting from not shaving for 48 hours, acne, seborrhea, obesity and increased body hair with suggestive male distribution of pubic hair. The clitoris was at the top limit of normal size, the labia majora pouting in appearance.

The occurrence of obesity in our group of patients was common. Historically, it often appeared that the hirsutism antedated the onset of overweight, and one is prompted to consider the obesity as representative of the overeating associated with psychological disturbance following the appearance of the abnormal hair, rather than a metabolic anomaly *per se*.

Complete radiographic studies of the skull and skeletal system of 29 of our patients failed to reveal an osteoporosis or other defect.[3] Complete analyses of the blood electrolytes have failed to show any abnormality. Complete blood counts have been normal. In a few patients recently studied, fasting eosinophile counts have been normal.

In 29 patients, we performed studies utilizing the glucose-insulin tolerance test.[3] Five of the group studied were frankly diabetic. Of the remainder, the results were abnormal in fourteen. We are at present unaware of the significance of this finding, but it must be pointed out that those patients who showed abnormal tests were usually obese, which may be a modifying factor in interpreting the results, since obesity is frequently associated with abnormal glucose tolerance.

Estimations of urinary 17-ketosteroids have been performed in 45 patients. The values range from normal to slightly higher values, a finding reported by others.[14] In the few instances in which 17-ketosteroids have been fractionated, there has been no increase in the beta fraction in idiopathic hirsutism.[3, 14] Recently, it has been claimed that there is a rhythmic increase in 17-ketosteroid excretion in hirsute women, associated with ovulation, as contrasted with normal women.[15] It is likewise of interest that, under conditions of stress, the idiopathically hirsute woman has an increase in 17-ketosteroid excretion to a point approaching that of the normal male.[16] From the meager data available, the excretion of 11-oxysteroids is normal.[17, 18] There is, however, an apparent increase in the ratio of androgens to estrogens excreted in the urine of these patients.[19]

Hirsutism may also be transient. Although somewhat unusual, transient hirsutism may accompany pregnancy. In two such cases, the abnormal hair began to disappear in the third trimester of pregnancy and, in the other, abnormal hair began to disappear in the post-partum. It is of interest that, in one case, the patient's mother had suffered an identical experience. The hormonal studies which were performed in these two instances showed no marked deviation from the normal.[3]

Transient hirsutism may also accompany severe thermal burns, and it has been observed during severe starvation. Occasionally, it may occur during therapy with dilantin and testosterone. This latter complication is being seen with increasing frequency, concomitant with the wide-spread use of large doses of testosterone in the palliative treatment of metastatic breast cancer.

The treatment of hirsutism is not satisfactory. When a demonstrable lesion is discovered, *i.e.*, a tumor, its removal will often reverse the masculinization. Even so, the hirsutism may be the last sign to disappear, if then. Idiopathic hirsutism is even more discouraging therapeutically. In our experience, endocrine preparations have been useless. Usually, the patient herself will resort to shaving, bleaching, tweezing, or depilatories. X-ray epilation is to be decried because of the danger of carcinogenesis. Electrolysis is perhaps most effective, but it is somewhat tedious, expensive, and not universally reliable.

Summary and Discussion

Hirsutism is defined as abnormal hairiness and is usually a clinical problem confined to the female. In a small percentage of cases, a causative lesion in the adrenal cortex, anterior pituitary, or ovary can be demonstrated. In the vast majority of cases of the so-called "idiopathic" hirsutism, no such organic lesion is apparent.

The term "idiopathic" is always an admission of ignorance of the basic processes involved. In these latter "idiopathic" cases, there is a strong suggestion of genetic factors, either familial or racial. Although this may be the entire explanation, there is some suggestive evidence from 17-ketosteroid determinations* and other assays (*supra vide*) that there may be mild adrenal-cortical hyperfunction. The occurrence of transient or permanent "idiopathic" hirsutism during pregnancy, at puberty, after burns, stravation, and similar stress, times in which adrenal cortical activity is known to be increased, further suggests an associated adrenal hyperfunction.

In the author's opinion, there remain to be studied, in "idiopathic" hirsutism, several further possibilities: (1) an inherited defect in the intermediary metabolism of sexual steroids from the parent substance cholesterol; (2) possible faulty conversion of normally estrogenic steroids to closely related androgenic substances; and (3) an abnormal, perhaps genetic, susceptibility of the hair follicle to a normal hormonal complex (increased end-organ response). The possible existence of steroids which specifically stimulate hair follicle growth is another unsolved problem. Obviously, much more careful study is necessary before any of these theses may find a sound and demonstrable basis.

Bibliography

1. Danforth, C. H. 1925. Arch. Dermat. & Syph. **12:** 380.
2. Albright, F. 1942–43. The Harvey Lecture Series. **38:** 123.
3. Bissell, G. W. & R. H. Williams. 1945. Ann. Int. Med. **22:** 773.
4. Talbot, N. B. & A. M. Butler. 1942. Jr. Clin. Endocrin. **2:** 724.
5. Calloway, J. L., J. F. Wortham, E. H. Hamblen, & A. A. Salmon. 1949. Arch. Derm. & Syph. **60:** 528.
6. Leyton, O., H. Turnbull, & A. Bratton. 1931. Brit. Jr. Path. & Bact. **34:** 635.
7. Horrax, G. 1936. Arch. Neurol. & Psych. **35:** 215.
8. Reich, N. E. & J. B. Reinhardt. 1948. Arch. Derm. & Syph. **57:** 725.
9. Epstein, E. 1947. Wein. Med. Wochenschrift **97:** 520.
10. Saphir, O. 1939. Am. Jr. Obs. & Gynec. **37:** 1008.
11. Geist, S. & J. Gaines. 1942. Am. Jr. Obst. & Gynec. **43:** 975.
12. Schwartz, J. H. 1942. Psychiat. Quarterly **16:** 281.
13. Schroeder, H. 1950. Personal communication.
14. Escamilla, R. F. 1949. Ann. Int. Med. **30:** 249.
15. Koets, P. 1949. Jr. Clin. Endocrin. **9:** 745.
16. Forbes, A. P., E. C. Donaldson, E. C. Reifenstein, & F. Albright. 1947. J. Clin. Endocrin. **VII:** 264.
17. Venning, E. H. & J. S. L. Browne. 1949. Ann. N. Y. Acad. Sci. **50**(6): 627.
18. Talbot, N. B., F. Albright, A. N. Saltzman, A. Zygmuntowicz, & R. Wixom. 1947. J. Clin. Endocrinol. **7:** 331.
19. Glass, S. J. & H. C. Bergman. 1938. Endocrinology **23:** 625.

---

* It should be remembered that determination of 17-ketosteroid excretion is not a parallel measure of androgenicity, since some compounds contributing to the 17-ketosteroid complex, as assayed chemically, are biologically inert (etoicholanol-3(a)-17-one, for example). As Venning and Browne have pointed out, however, "The adrenal precursors of these substances have not been identified, but they are considered to be associated with the androgenic function of the adrenal."[17]

Discussion of the Paper

Doctor James T. Wortham: I have been asked to incorporate a discussion of the endocrine therapy of hirsutism with a discussion of Dr. Bissell's excellent review of hirsutism in general. It would be difficult to say anything that has not been mentioned already by Dr. Bissell. However, a few observations may further clarify the problem of excessive hair growth in women.

It seems almost impossible to divorce hirsutism from masculinization in the minds of laymen and, to a great extent, even in scientific minds. Nevertheless, hirsutism is a poor index of masculinization and of androgenicity in the individual patient. Some female patients with adrenal or ovarian neoplasia who are generally severely masculinized may not have striking amounts of excessive body hair. It has been my experience that women with essential hirsutism, that is hirsutism without demonstrable endocrine disease, will have, as a general rule in severe cases, more excess hair than women with neoplastic production of excessive androgens.

The first step in the endocrine therapy of hirsutism in women, then, is to demonstrate, or rule out, a masculinizing pathological process. If one accepts the premise that hair growth is a poor index of excessive androgen levels in the individual patient, then what procedures will serve to measure true hyperandrogenism? Clinical evaluation is most reliable. The state of muscular development, the timbre of the voice, the texture of the skin, the status of the clitoris, the state of preservation of gynecoid fat padding and breast development, and the status of preservation of gynecic function are the observations of greatest value. One thing has become evident: a pathological masculinization is not compatible with normal ovarian function. Qualification of ovarian function with menstrual calendars, basal body temperature graphs, and endometrial biopsy data will frequently, if the data are normal, rule out masculinization.

Colorimetric assay of 17-ketosteroids is the laboratory procedure most commonly used to prove or disprove excessive androgen production. It is a poor index indeed. It is a chemical method and does not indicate biologic androgenicity of these urinary degradation products of androgen catabolism. As Dr. Bissell has mentioned, patients with markedly virilizing ovarian tumors most often excrete a normal number of milligrams of 17-ketosteroids. Yet these androgens, which may well be abnormal in their fractional content of various ketosteroids, are obviously very potent biologically. Some of the very high 17-ketosteroid levels, however, have been found in patients with virilizing ovarian tumors. Methods of bioassay of androgens are not readily available, and too few androgen-estrogen ratio studies are available to arrive at any conclusions.

We feel, therefore, that clinical evaluation of the patient is the most reliable index of masculinization. Borderline patients are a little more difficult. Psychic problems secondary to a disfiguring hirsutism often result in over-eating and obesity. Obesity alone may suppress normal ovarian cyclicity. Yet even in such cases, if clinical assay yields no evidence of masculinization or defeminization save for the hirsutism, differentiation of essential hirsutism is not difficult. The obese, hirsute woman with infrequent menses and, frequently, a diabetic glucose tolerance curve usually retains only her hirsutism when adequate reduction in weight is obtained.

Patients with masculinizing syndromes are usually treated surgically or occasionally roentgenologically. Success depends upon the type of neoplasm. Even if successful, the hirsutism component of the virilization corrects itself slowly. The woman with excess hair but without virilization is often treated hormonally.

One wonders why such a compulsion exists to alter the hormonal milieu of hair follicles, when it has been observed that no other structure or organ system of the body has reacted to demonstrate excess male hormones in most of these patients. It is now obvious that hormonal influences are only one segment of those factors governing hair follicle activity. Genetic and racial factors which will produce hair growth which might be misinterpreted as endocrinopathic are well recognized. Few studies of the factors by which hair follicles react excessively to normal hormonal environment have been done. Undoubtedly, genetic and racial factors are of most importance in this phenomenon of local tissue hyperreactivity, but this needs further clarification.

Hormonal therapy of essential hirsutism is, therefore, purely empiric. It has been impossible to prove that excess hair growth *per se* represents endocrine imbalance, despite our continuous compulsion to try to do so.

Most frequent efforts have been toward rendering the hormonal environment of hair follicles more strongly estrogenic, by giving various estrogens by inunction or parenterally. This seems rather illogical when we remember that most essential hirsutism develops during the epoch of a woman's life when her hormonal balance becomes more positively estrogenic, during pubescent years. Poor results are noted on the hair growth, as many reports will indicate. Extremely unwelcome complications of this estrogen therapy include: disruption of ovarian cycles by acyclic administration of estrogens; undesired sterility by suppression of ovarian function by therapy; and induced aberrations of bleeding which frequently becomes excessive to the point of debilitation, frequently requiring hemostatic curettement of the uterus and cyclic steroid therapy to re-establish normal ovarian function.

No other steroidal fraction has proven to be of any considerable value in the treatment of essential hirsutism. Perhaps we need to change our perspective, realize that excess hair growth does not necessarily indicate an abnormal androgen-estrogen ratio, and continue with the studies of the other multiple factors affecting hair growth which have been so well presented in this monograph. At the present time, it is impossible to incriminate the endocrine glands or their metabolic systems in the production of essential hirsutism. Nevertheless, this challenge will not go unanswered as we learn more about steroid chemistry and hormonal methods.

THE ROLE OF THE PILIARY SYSTEM IN CANCER OF THE SKIN

By N. Chandler Foot
Professor Emeritus of Surgical Pathology, Cornell University Medical College, New York, N. Y.

The title assigned to this all-too-brief discussion indicates that it should adhere very closely to the possible inter-relationship of cancer of the skin with pilar follicles. We know that there are nonmalignant tumors over which there is an excessive, though not neoplastic, growth of hair, while others consist largely of distorted hair follicles. The so-called "trichoepithelioma," or "epithelioma adenoides cysticum," is an example of the latter. It may be closely associated with tumors of dermal adnexa other than hairs, such as the solid hidradenomas of sudoriparous glandular origin. That such a tumor might be transformed into the growth which will be the main topic of this discussion is a possibility, though not too great a probability.

Cancer of the skin comprises three types of tumor: one of them is an inherent part of the skin and arises from it, one (usually called "basal cell epithelioma") is of disputed origin, and the last type comprises secondary, or metastatic growths, which do not properly concern us here. The epidermoid, squamous, or genuinely dermal carcinoma is also outside our scope, as it does not have any connection with distorted hairs or piliary growth. The basal cell epithelioma is the tumor which, of all dermal neoplasms, has a probably close relationship to hair follicles.

This tumor was originally described about 50 years ago by Krompecher, who gave it the name "Basalzellenkrebs." He believed that it arose from an ingrowth of basal cells in the epidermis which took the form of invasive plugs of these elements, which spread into the corium, eroded normal structures in its path, and broke down and ulcerated when it overshot its service of supplies. Many years later, Mallory advanced the view that the tumor originated not in basal cells as such, but rather from piliary follicles. His theory flourished among his pupils in the vicinity of Boston, but found rather sterile ground elsewhere, as pathologists were unable to trace any direct connection between the complexes of neoplastic cells and the adult hair follicles. He had pointed out that there were abortive hair shafts developing at the center of some of the aggregations of rather simple basal cells. Then came the concept of the solid hidradenoma, a tumor developing from perverted sudoriparous glands and showing an architecture strikingly similar to that of the basal cell epithelioma. These two observations have a direct bearing upon the possible origin of the tumor we are discussing. Walter Lever of Boston and the author investigated the matter quite independently and coincidentally and came to essentially the same conclusions. Welton, Elliott, and Kimmelstiel have checked over our ideas and substantiated them in large measure. There is, however, a large group of pathologists who still adhere to the classic concepts of Krompecher.

By means of tridimensional sectional reconstruction, and by utilizing a very early example of the tumor excised when it was still but one millimeter or so in diameter, it was possible to draw conclusions as to its development.

It was immediately evident that it arose, often in multiple foci, from the necks of hair follicles where there is an abundance of poorly differentiated basal cells. By multiplication, these produce papillary downgrowths into the underlying corium and these then balloon out into fantastic gourd-shaped masses suspended from pedunculated isthmuses. It seems, on analyzing the morphology of the cells, that they show a tendency to produce pilar follicles, in most instances, and sebaceous and sudoriparous complexes in a few. The pilar follicles thus generated, however, differ from the adult hair matrix. They show little differentiation into layers, and their general line of development closely follows that of embryonal pilar primordia. Thus, it would seem that Mallory was correct in the use of the term "hair matrix carcinoma," but incorrect in the assumption that an adult hair matrix was involved.

Tumors developing from processes that recapitulate the embryonal evolution of hair follicles from certain of the basal cells situated near the outlet of adult follicles bear out the term "basal cell epithelioma," as they are certainly basal and epithelial, but the term is too general and indicates that any random basal cell might produce the tumor for which I have proposed the name *adnexal carcinoma*. Many dermatologists still maintain that any basal cell in the epidermis can develop this tumor, but if that is true, why does the resulting growth follow the morphogenesis of the embryonal hair follicle, and why is it almost invariably attached to the adult hair follicle?

Critics of this idea usually base their conclusions upon evidence obtained from the examination of single sections of large, well-developed tumors or, at best, from short series of sections. One may often find papillary downgrowths of apparently nonspecific basal epidermal cells in several successive sections, but, if one continues to cut further and then constructs a tridimensional model, it will be found that the peduncular root of the tumor, from the neck of the piliary follicle, will ultimately be located several sections deeper in the paraffin block. These pedicles are often insignificantly small, but they are there, although quite overshadowed by the burgeoning extension from the deeper portions of the growth.

Adnexal carcinoma is a skin cancer or rodent ulcer, but its actual clinical behavior, when it is excised in its incipiency, is very mild and belies the grimness of its popular name. Only when permitted to grow unmolested for years and to ulcerate extensively does it become truly malignant in the clinical sense of the term, and it very rarely metastasizes or spreads to other parts of the body. Therefore, some pathologists wish to do away with the term "carcinoma" entirely in connection with this growth. At the present time, it is recognized before it is more than a quarter of an inch in diameter and is widely removed at that time. Thus, it is little more menacing than an ordinary cutaneous blemish. In rural districts, on farms and on ranges, patients are less conscious of cosmetic appearance and allow these tumors to grow to relatively large proportions. They are frequently encountered in areas where the sun is bright and its ultraviolet rays relatively unimpeded by atmosphere and smog.

Several histologic subtypes may be recognized, but that would be of little interest here. A classification of these appears to have little bearing on the prognosis, or ultimate outlook. They all act much the same. Perhaps the best way of stressing the point of adnexal origin is by reference to photographs of sections and reconstructions made before coming to the conclusions just set forth.

These lantern-slides were all taken from negatives used to illustrate the article "Adnexal Carcinoma of the Skin."[5] A typical tumor complex is shown in Figure 1 of that article and the somewhat similar pattern of solid hidradenoma (for comparison) by Figure 5. Figure 18 pictures the balloon-like downgrowth of the tumor from the epidermis and represents one of many sections used in making the tridimensional model depicted in FIGURES 9 and 10. Somewhat schematic representations of pilar and sudoriparous anlagen are shown in Figures 14, 15, and 16. These were reproduced from the first edition of the "Textbook on Histology," by Maximow and Bloom, with the kind permission of Dr. Bloom and his publishers, the W. B. Saunders Company. As for the technique of constructing tridimensional models, the following method was employed.

Serial sections were made from a minute tumor, likely areas were chosen for reconstruction, and images of these sections were projected onto ordinary filing cards in succession, until many sections had been traced in outline on the cardboard. The outlines were then cut out with scissors and the resulting paper silhouettes glued together, until a tridimensional phantom had been built up. This was smoothed off and painted. The enlargements were gauged so that the thickness of the cardboard represented that of a paraffin section multiplied about 40 times. When the magnification was increased to 70 or 80 diameters, two thicknesses of cardboard were employed for each serial section, thus maintaining the ratio.

It would be well once more to enunciate my criticism of the term "basal cell epithelioma". It is simply too general to be specifically descriptive. There are more dermatogenic than pilogenic basal cells in the epidermis, and the term "epithelioma" merely implies any epithelial tumor and fails to indicate the possibility of cancerous traits which the neglected adnexal carcinoma certainly possesses.

References

1. KROMPECHER, E. 1900. Der drüsenartige Oberflächenepithelkrebs. Carcinoma epitheliale adenoides. Beitr. z. path. Anat. u. z. allg. Path. **21:** 1.
2. KROMPECHER, E. 1903. Der Basalzellenkrebs. G. Fischer. Jena.
3. MALLORY, F. B. 1910. Recent progress in the microscopic anatomy and differentiation of cancer. J. A. M. A. **55:** 1513.
4. MALLORY, F. B. 1914. Principles of Pathologic Histology: 371. W. B. Saunders. Philadelphia.
5. FOOT, N. C. 1947. Adnexal carcinoma of the skin. Am. J. Path. **23:** 1.
6. LEVER, W. F. 1948. Pathogenesis of benign tumors of cutaneous appendages and of basal cell epithelioma. Arch. Dermat. & Syph. **57:** 679.
7. LEVER, W. F. 1948. Histopathology of the Skin. J. B. Lippincott. Philadelphia.
8. WELTON, D. G., J. A. ELLIOTT, & P. KIMMELSTIEL. 1949. Epithelioma; clinical and histologic data on 1,025 lesions. Arch. Dermat. & Syph. **60:** 277.

MONOGRAPHIC PUBLICATIONS

OF

THE NEW YORK ACADEMY OF SCIENCES

(Lyceum of Natural History, 1817–1876)

(1) The Annals (octavo series), established in 1823, contain the scientific contributions and reports of researches, together with the records of meetings of the Academy. The articles which comprise each volume are printed separately, each in its own cover, and are distributed immediately upon publication. The price of the separate articles depends upon their length and the number of illustrations, and may be ascertained upon application to the Executive Director of the Academy.

Current numbers of the Annals are sent free to all Members of the Academy desiring them.

(2) The Special Publications, established in 1939, are issued at irregular intervals as cloth-bound volumes. The price of each volume will be advertised at time of issue.

(3) The Memoirs (quarto series), established in 1895, are issued at irregular intervals. It is intended that each volume shall be devoted to monographs relating to some particular department of Science. Volume I, Part 1, is devoted to Astronomical Memoirs, Volume II to Zoological Memoirs. No more parts of the Memoirs have been published to date. The price is one dollar per part.

(4) The Scientific Survey of Puerto Rico and the Virgin Islands (octavo series), established in 1919, gives the detailed reports of the anthropological, botanical, geological, paleontological, zoological, and meteorological surveys of these islands.

Subscriptions and inquiries concerning current and back numbers of any of the publications of the Academy should be addressed to

EXECUTIVE DIRECTOR
The New York Academy of Sciences
2 East Sixty-third Street
New York 21, N. Y.